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OBSERVATIONS ON TWO MALARIA VECTORS AND DISTRIBUTION RECORDS OF OTHER *ANOPHELES* IN THE STATES OF BAHIA AND SERGIPE, BRAZIL*

BY
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(Received for publication 23 November, 1931)

There has been much confusion in the literature in regard to the nomenclature of the malaria vectors of Brazil. Chagas is supposed to have demonstrated natural infection of both *Anopheles argyritarsis* and *A. tarsimaculatus* in 1904 and 1905. Boyd (1926) dissected mosquitoes near the Federal Capital in the State of Rio de Janeiro, and reported natural infections in *A. argyritarsis*, *A. brasiliensis*, *A. tarsimaculatus* and *A. oswaldoi*. Godoy and Pinto in 1923 said that they found *A. (Cellia) brasiliensis* infected in nature near Campos in the State of Rio. Later Root (1929) pointed out that the species referred to by Brazilian entomologists as 'brasiliensis' and 'argyritarsis' are really *A. albitarsis*, and Boyd (1930) referred to the mosquitoes dissected by him in Brazil as *A. albitarsis* and *A. tarsimaculatus* only. Covell (1927) included both 'argyritarsis' and 'brasiliensis' under *A. albitarsis*.

Godoy, Lobo, and Oswaldo Cruz, Jr. (1930), obtained experimental infection of *A. tarsimaculatus* with *Plasmodium vivax*. They also dissected house-caught *Anopheles*, and reported a natural infection of the salivary glands in one single specimen of *A. albitarsis*. However, this infected *A. albitarsis* was found at Estrella in the State of Rio de Janeiro, and it may possibly have been *Anopheles darlingi* rather than *A. albitarsis*, for Estrella lies between Iguassú and Magé, at both of which Root (1926) found *A. darlingi*.

While working near Porto das Caixas (State of Rio de Janeiro), Root (1926) discovered *A. darlingi*. He later met with it at four other localities in the same region, but was not able to collect any information as to its relation to malaria transmission. The first

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation. (From the Yellow Fever Laboratory, Bahia, Brazil.)

dissections of *A. darlingi* were made by Davis (1931) at Belém, State of Pará, where he found a natural infection rate of 22 per cent. Davis was able to do additional dissections of *A. darlingi* at França in the State of Bahia (1931), and he obtained a natural infection rate there of 28.7 per cent. Both at Belém and at França he found salivary gland infections as well as midgut infections. He therefore concluded that *A. darlingi* appeared to be a very dangerous vector. He also pointed out that the 'argyritarsis' mentioned by Boyd and himself was probably a mixture of *A. albitarsis* and *A. darlingi*, for both of these mosquitoes occur in the region where Boyd's dissections were made.

We have recently had the opportunity of doing some additional dissections on both *A. albitarsis* and on *A. darlingi*, and although the work is neither complete nor thorough the results are given herewith because of the great importance of these two mosquitoes in this part of Brazil. On March 26, 1931, Dr. D. B. Wilson, of the Yellow Fever Control Service, informed us that one of his guardas had been finding many *Anopheles* in houses in one section of the city of Bahia. Between March 27 and April 10, 423 adult *A. albitarsis* were captured in houses in that quarter. All of them were identified by us as being of this species, but because of the previous confusion in regard to the identification of this mosquito, thirty-eight mounted specimens and a considerable number of unmounted ones were sent to Dr. F. M. Root at Baltimore. Dr. Root confirmed the identifications as *A. albitarsis*, but pointed out that most of the specimens that we had sent to him were intermediate in colouration between the 'albitarsis' of the coastal lowlands near Rio de Janeiro and the 'brasiliensis' from the type locality, Lassance. There were also a few individuals indistinguishable from the type of 'albitarsis,' which he had found so abundant on the 'llanos' of Venezuela near the Orinoco River. Dr. Root was in favour of dropping the varietal name 'brasiliensis' altogether, and simply calling all the different forms *albitarsis*.

We dissected 240 midguts successfully and found oöcysts in fourteen, or 5.8 per cent. Most of the small cysts had large granules of coarse black pigment suggesting that they were young zygotes of *Plasmodium falciparum*. The maximum number of oöcysts on any stomach was four, and frequently only one was seen. But this

is not surprising, as no unusual prevalence of malaria had been reported in Bahia. We dissected forty-eight salivary glands without finding any sporozoites. Presumably if more had been examined, gland infections also would have been demonstrated.

From March 7 to 9, 1931, we investigated an epidemic of suspected yellow fever in the small town of Itapira on the Rio de Contas, south of Bahia. Instead of yellow fever, we found a severe epidemic of malaria. Both *P. vivax* and *P. falciparum* were identified in blood films from patients. Mosquito captures were made both inside houses and outdoors at sunset with a horse as bait. Seven species of *Anopheles* were taken. Not many of the *Anopheles* captured in Itapira could be transported to Bahia alive, but the results of the dissection of the few that arrived alive are given herewith:

	Number of stomachs infected	Number of stomachs not infected	Total number of stomachs dissected
<i>A. darlingi</i> (house captures)	3	2	5
<i>A. darlingi</i> (horse bait captures)	0	5	5
<i>A. albitarsis</i> (horse bait captures)	0	5	5
<i>A. tarsimaculatus</i> (horse bait captures)	0	8	8

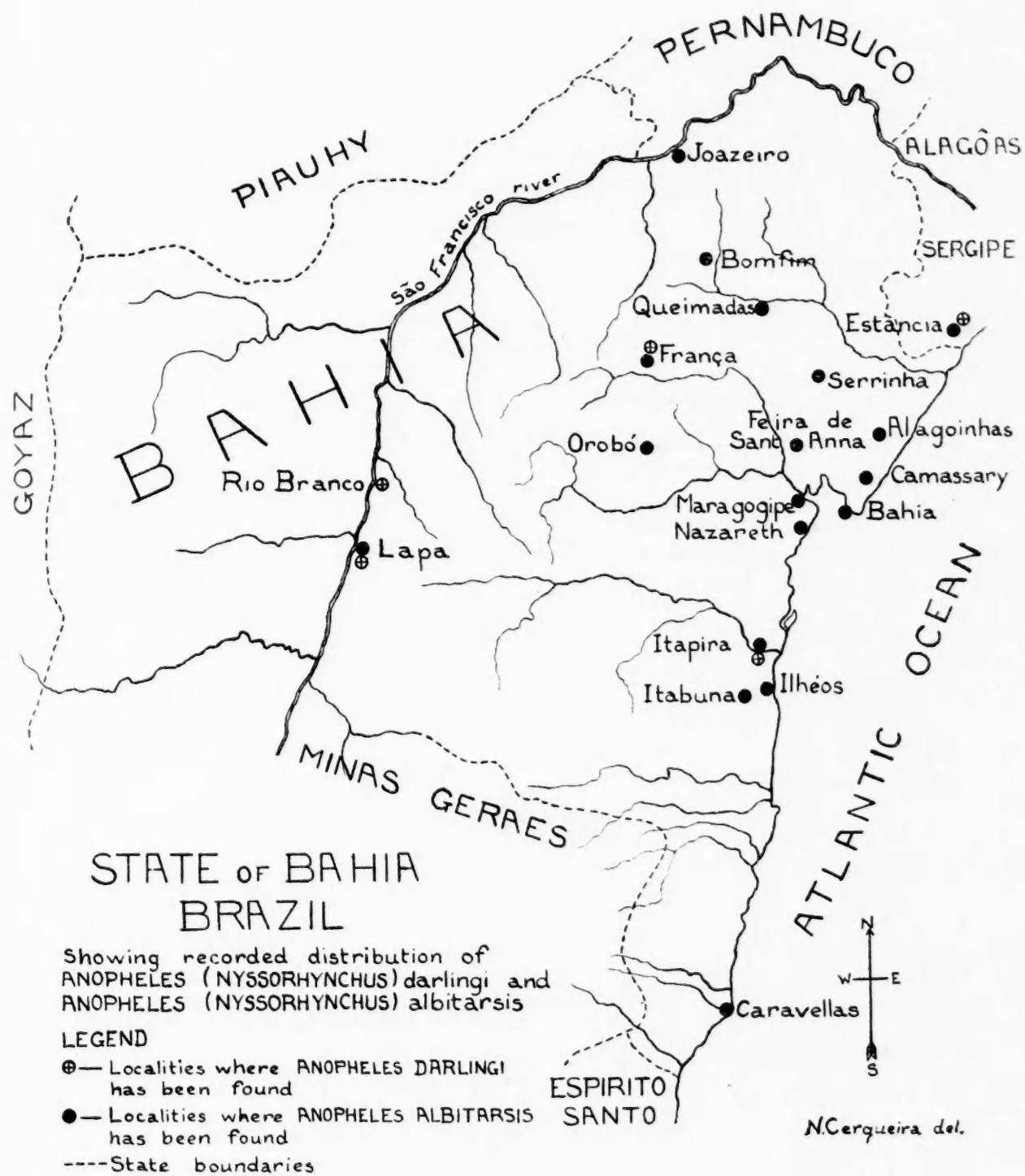
In Itapira, *A. darlingi* was the only *Anopheles* that was found in houses. One of the infected stomachs had 100 oöcysts and the other two had about ten oöcysts each. The number of oöcysts per stomach found in *A. darlingi* at Itapira was much larger than that found in *A. albitarsis* in Bahia; but in addition to the probable difference in the infectivity of the two species, there was a severe epidemic of malaria at Itapira when the dissections were made, whereas at Bahia there was no more malaria than occurs every year during March and April.

Anopheles have been captured in nineteen towns in the states of Bahia and Sergipe by various members of the yellow fever service, incidental to their other work. Although the records are scanty, they indicate a wide distribution of *A. albitarsis*, *A. tarsimaculatus*, *A. bachmanni* and *A. argyritarsis*. Up to the present we have not found *A. darlingi* in any of the coast towns, namely Bahia,

Maragogipe, Nazareth, Ilhéos or Caravellas. In the State of Rio de Janeiro, on the other hand, Root recorded it from the 'coastal plain region.' A table of the distribution records is given below as well as a spot map showing the records for *A. albitarsis* and *A. darlingi*.

Distribution records of *Anopheles* in the States of Bahia and Sergipe.

Name of town	By whom reported	<i>Anopheles albitarsis</i>	<i>Anopheles tarsimaculatus</i>	<i>Anopheles bachmanni</i>	<i>Anopheles argyritarsis</i>	<i>Anopheles darlingi</i>	<i>Anopheles strodei</i>	<i>Anopheles intermedius</i>	<i>Anopheles mediopunctatus</i>	<i>Anopheles fluminensis</i>	<i>Anopheles minor</i>	<i>Anopheles peryassui</i>	<i>Anopheles parvus</i>	<i>Anopheles bellator (cruzi?)</i>	<i>Stethomyia nitida</i>	<i>Chagasia fajardo</i>	Totals
STATE OF BAHIA																	
Alagoinhas	da Silva, per Kumm ...	+	+	+	+	+	5
Bahia	Davis and Shannon ...	+	+	+	+	+	+	...	+	+	...	+	+	...	10
Bomfim	Shannon	+	+	+	+	...	+	+	+	7
Camassary	Cerqueira, per Kumm...	+	+	...	+	+	+	5
Caravellas	Davis	+	+	+	+	+	5
Feira de Sant' Anna ...	Seraphim, per Kumm...	+	+	...	+	3
França	Davis and Seraphim ...	+	+	+	+	+	5
Ilhéos	Davis and Shannon ...	+	+	+	+	+	+	6
Itabuna	Seraphim	+	+	...	+	3
Itapira	Kumm and Seraphim ...	+	+	+	...	+	...	+	...	+	+	7
Joazeiro	Bião, per Shannon ...	+	1
Lapa	Bião, per Shannon ...	+	...	+	...	+	3
Maragogipe	da Silva and Seraphim	+	+	+	+	4
Nazareth	da Silva, per Kumm ...	+	+	+	+	+	5
Orobó	Seraphim	+	+	+	+	4
Queimadas	Kumm	+	1
Rio Branco	Bião, per Shannon	+	1
Serrinha	Kumm	+	1
STATE OF SERGIPE.																	
Estancia	Shannon	+	+	+	+	+	...	+	+	7
Totals		18	14	12	12	5	1	5	2	1	6	2	1	2	1	1	83



SUMMARY

1. A natural malaria infection rate of 5.8 per cent. was found in the midguts of 240 *A. albitarsis* captured in the city of Bahia, Brazil.
2. *A. darlingi* with numerous oöcysts in the stomach were demonstrated at Itapira, the third place in Brazil where this mosquito has been found naturally infected.
3. The demonstration of natural infection in *A. albitarsis* confirms the work of Boyd, Davis and others, near Rio de Janeiro.
4. *A. albitarsis* and *A. tarsimaculatus* appear to be the commonest *Anopheles* in the State of Bahia.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the help received from Dr. F. M. Root of the Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland, in the identification of the *A. albitarsis* dissected in Bahia. Dr. Root also checked the identifications of the mosquitoes collected in Itapira.

Thanks are due to Dr. N. C. Davis and to Mr. R. C. Shannon for permission to quote their distribution records, and also to Dr. José Seraphim, Jr., who kindly allowed us to examine his collections from certain localities in the State of Bahia and to quote his findings in those places.

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THE SECRETORY GLANDS OF THE CERCARIAE OF *S. HAEMATOBIIUM* AND *S. MANSONI* FROM EGYPT

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When Leiper (1915-1918) investigated the life-history of *S. haematobium* and *S. mansoni* in Egypt and published his discoveries and observations, he gave no detailed account of the anatomical features of the cercariae of these schistosomes. He considered that only experimental infection with mammals would determine the systemic position of such cercariae. Iturbe (1917) described three pairs of glands in the cercaria of *S. mansoni*. Faust (1919) described three pairs of mucin glands in the cercaria of *S. haematobium* in specimens from Natal. The glands began in the region of the acetabulum on either side and were said to empty their contents by mucin gland ducts at the outer margin of the oral sucker. The mucin glands had loosely scattered granules in their cytoplasm and large nuclei, the granules being acidophilic. No mucin glands other than the above mentioned three pairs were found.

In specimens of the cercaria of *S. mansoni* from Caracas and Venezuela which he examined, the mucin glands consisted of two pairs of cells of the granular type, and in addition, four pairs of a non-granular type, somewhat smaller and surrounding the granular cells, were described. The ducts were decidedly heavier than in the South African species.

Later Faust (1920) stated that, while probable differences in size and shape obtain in the case of the cercariae of *S. haematobium* and *S. mansoni*, which allow of their differentiation from the cercaria of *S. japonicum*, the number and type of mucin glands was the most dependable basis of diagnosis. Thus the larvae of *S. haematobium*

had only three pairs of glands with a corresponding number of ducts opening strictly lateral to the orifice. The glands had small nuclei and give a simple acidophilic reaction. On the other hand the larvae of *S. mansoni* had six pairs of glands, with an equal number of ducts which were arranged around the orifice dorso-laterally in the form of two compressed crescents. Moreover, this species had the glands differentiated into two types: two of the glands are acidophilic with large nuclei, while four give a basophilic reaction and had small nuclei.

Manson-Bahr and Fairley (1920) dissected the livers of infected snails of *Bulinus contortus* and *B. dybowskii* and *Planorbis boissyi* in Egypt, and obtained the cercariae of *S. haematobium* and *S. mansoni* from these by teasing. In describing the anatomy of these cercariae, they stated that on each side of the ventral sucker lie the 'poison cells' or salivary glands, each of which contained a well-marked nucleus, and from these individually a duct could be distinguished passing forwards into the mouth. The number of these salivary mucin glands varied in the two species in the specimens they examined. Thus in *S. haematobium* cercaria they found three pairs of large clear cells with acidophil protoplasm and clear-cut nuclei. In *S. mansoni* cercaria there were six pairs of glands altogether, two of which were large and clear with large nuclei, and four were small and granular with correspondingly smaller nuclei.

Khalil (1922) commented that no reliable account of the anatomy of the cercaria of *S. haematobium* was then available. In order to study the anatomy of the cercaria of *S. mansoni*, he examined a large number of living infected *Planorbis boissyi* snails which were sent to him in London by Professor Leiper from Egypt. They were collected from the same site where *S. mansoni* cercariae were first discovered. He used for the secretory glands the name 'peri-acetabular glands' proposed by Milton. These glands overlapped each other and their outline was difficult to make out in many specimens, though occasionally in some cercariae it was quite clear; the same was true of the presence and definition of the nuclei. He attributed this to the accumulation of secretion in the glands.

Regarding the number of glands in any one cercaria, his work revealed the presence of five pairs only, and the evidence of this finding was corroborated by the concomitant presence of five pairs

of ducts and five pairs of spines. These glands were anatomically divisible into two groups :

1. An anterior group composed of one pair of cells on either side. These were large glands with bulbous posterior extremities and tapering anteriorly to fine ducts. They were situated just in front of the ventral sucker. Their protoplasm was very granular; the granules were large and this gave the glands an appearance which differed from that of the posterior group. The granular appearance could also be seen throughout the length of their ducts. In stained sections these granules disappeared leaving a clear protoplasm, and he suggested that the granules might be soluble in the reagents used in the staining processes. The nucleus was large, vesicular and situated in the bulbous portion; there was also a large nucleolus.
2. The posterior group of peri-acetabular glands consisted of three pairs. They were much smaller glands and situated principally posterior to the ventral sucker. The two anterior glands were situated side by side, and the third posterior to both. Their protoplasm was finely granular and the nuclei were vesicular with large nucleoli.

Cawston (1922), comparing the various schistosomes found in South African snails, stated that in the cercaria of *S. haematobium* there were three acidophilic pairs of mucin glands with large nuclei. In the cercaria of *S. mansoni* there were two acidophilic pairs of mucin glands with large nuclei and four basophilic pairs with small nuclei.

Bettencourt and Da Silva (1922) described in the cercaria of *S. haematobium* three pairs of glands, with large acidophil granules in the cytoplasm, occupying nearly the whole of the posterior portion of the body.

Blacklock and Thompson (1924), in a paper published in May of that year, stated that the posterior two-thirds of the body of the cercaria were almost entirely occupied by pairs of unicellular glands, five on each side, each with a single and definite nucleus. The protoplasm of the cells contained granules which were of large size in the anterior two pairs, and of small size in the posterior three pairs of glands. The two anterior pairs of coarsely granular

salivary glands were large and flask-shaped lying just anterior to the ventral sucker; the three posterior pairs of finely granular salivary glands lay behind the anterior two pairs and were smaller in size and overlapped each other. They found that the affinity of the glands for stains was not constant, and they suggested that this variability of staining capacity might be due to variations in the secretory activity of the glands.

Their cercaria was identified as being that of *S. haematobium* on the following grounds.

1. The incidence of *S. haematobium* in the human host, i.e., epidemiological data.
2. The presence of naturally infected *Physopsis globosa* (Morelet).
3. Experimental infection of these snails.
4. The positive results of experimental infection in laboratory animals.

In a subsequent paper written in June (1924), these authors stated that they had now had the opportunity of seeing Khalil's paper (1922), in which he gave a description of the salivary glands of the cercaria of *S. mansoni*. Commenting upon the similarity of Khalil's and their own description of the glands of *S. mansoni* and *S. haematobium* respectively, they drew attention to the fact that the most recent studies disclosed no constant morphological character nor staining reaction by which the cercaria of *S. haematobium* and *S. mansoni* could be distinguished.

Bettencourt and Da Silva (1925) immediately issued a paper in a pamphlet form, specifically a criticism of the work of Blacklock and Thompson (1924) on the anatomy of the cercaria of *S. haematobium*. They stated that, after seeing the papers of these authors, they had repeated their former work, which was published in 1922, and that the repetition left them in no doubt that their own previous results were amply confirmed. They reiterated their conclusions and stated positively that in *S. haematobium* there were only three pairs of unicellular glands, all of one type, disposed symmetrically, with a protoplasm rich in acidophilic granulations, and each containing a large basophilic nucleus and a nucleolus. From these glands, ducts of the same number ran forwards and terminated at the anterior sucker.

These results were based on the examination of the cercaria

from naturally infected *Planorbis metidjensis*, the snail which, they affirmed, was beyond dispute the intermediate host of *S. haematobium* in Tavira. On infecting mice with such cercariae, adult males and females of *S. haematobium* were recovered. After declaring the confirmation of their work by that of Faust, Manson-Bahr and Fairley, and the unmistakable certainty of their own cercaria being that of *S. haematobium*, they criticised the work of Blacklock and Thompson, casting doubt on the cercaria of these authors being that of *S. haematobium*.

Faust (1926) revised his former work on human schistosome cercariae and, after a careful study of material from South Africa and Palestine, and the preparation of fresh sections, stained with Delafield's haematoxylin and counterstained with Best's alum-carmin, he declared: 'The writer is able to state that he is in full agreement with Blacklock and Thompson with respect to the number of glands in the cercaria of *S. haematobium*.' He also found that the glands were of two types: two pairs with large nuclei and granular acidophilic cytoplasm, and three pairs with a basophilic reaction. He stated in commenting upon his previous observations on the secretory glands of *S. haematobium*, that freshly prepared *toto* mounts of well-preserved specimens of the cercaria of *S. haematobium*, with or without eosin counterstain, give a similar picture to that which he first observed and described as the one prevailing in the cephalic secretory glands of the cercaria of *S. haematobium* from *Isidora africana* from Natal, namely, three pairs with granular acidophilic cytoplasm. However, *toto* mounts that have been allowed to 'age' for a year or more, when carefully studied, can be seen to possess two types of glands, an anterior group of two pairs and a posterior group of three pairs. He thus advises, when dealing with such schistosome cercaria, to use Best's alum-carmin counterstain on material previously sectioned and stained with haematoxylin or other fundamental tissue stain, in order to bring out this existing difference between the two types of cephalic glands now known to be present in this species of cercaria.

He also dissected out the cercariae from some of the infected livers of *Planorbis guadeloupensis* from Venezuela, and then stained and mounted them entire. In other cases sections of the liver containing the cercaria were made and specifically stained for

study with Delafield's haematoxylin, and counterstained with Best's alum-carmin, which gave him very satisfactory results. He also re-studied the original slides from the Venezuelan material which were made from the liver of *P. guadeloupensis* containing the cercariae of *S. mansoni*. His findings agreed in every respect with those of his earlier study (1919), i.e., that the cercaria of *S. mansoni* has two pairs of acidophilic and four pairs of basophilic glands, with an equal number of gland ducts. He commented that Khalil (1922) claimed to have found only five pairs of such glands in a larva which he designated as 'the cercaria of *Schistosomum mansoni*' secured from *Planorbis boissyi* near Cairo, and that, although the material was taken from the same species of snail which Leiper (1915) had demonstrated to harbour the cercaria of *S. mansoni* near Cairo, there was no experimental proof that he was dealing with this species of larva.

Referring to his own material he stated that it was collected from a known experimental source in a region in which only *Schistosomum mansoni* was known.

He concluded by stating that the three human schistosome cercariae, and also that of *S. bovis* and *S. spindalis*, could be distinguished from one another on the basis of the cephalic secretory glands, which are constant in number for each species and which have, at least in mature cercariae, specific acidophilic and basophilic properties which allow of their definite differentiation.

Faust (1930), in the first edition of his book on 'Human Parasitology,' again described in the cercaria of *S. haematobium* two pairs of anteriorly situated unicellular glands with granular contents and oxyphilic reaction, and three pairs of posteriorly situated unicellular glands with homogeneous contents and a basophilic reaction. In the cercaria of *S. mansoni* he described two pairs of anteriorly situated glands with granular acidophilic cytoplasm, and four pairs of posteriorly situated glands with basophilic slime contents.

The writer (1931) demonstrated afresh, in the cercaria of *S. haematobium* from Sierra Leone, the presence of five pairs of secretory glands: two anteriorly placed pairs about the level of the ventral sucker, large in size and coarsely granular, having oxyphilic granules, and three posteriorly placed pairs of small

size and finely granular or homogeneous and basophilic. Each nucleus in the two varieties of glands possessed a distinct nucleolus. The writer's results thus confirmed the findings of Blacklock and Thompson on the number and type of the secretory glands of the Sierra Leone cercaria of *S. haematobium*.

Professor D. B. Blacklock, under whom the writer was working in the Liverpool School of Tropical Medicine, advised him to work on the question of the secretory glands of the cercariae of *S. haematobium* and *S. mansoni* in Egypt during this summer. The necessity for approaching this problem arose from the fact that there is still a difference as to the number of these glands of *S. mansoni* cercariae in the findings of Faust and the work of Khalil, and that Manson-Bahr and Fairley, who worked out this subject on the Egyptian cercariae, upheld Faust in his results on *S. mansoni* cercaria. Moreover, since the work of Manson-Bahr and Fairley on the cercaria of *S. haematobium* in Egypt, in which they only found three pairs of secretory glands, no person has attempted to verify these results.

This piece of work was attempted by me last winter in the Liverpool School of Tropical Medicine, and Professor Khalil Bey was kind enough to send a batch of *Planorbis* and another batch of *Bulinus* snails from Egypt by air mail. The snails in these two batches were found to harbour no schistosome infection, with the exception of one *Bulinus* snail; the work was given up temporarily till the summer, during which season the incidence of infection in the snails rises.

TECHNIQUE

Batches of *Bulinus* and *Planorbis* snails from Marg, an endemic centre of schistosomiasis near Cairo, were collected weekly. The snails were identified, and put each in a test-tube with about 5 c.c. of tap water and kept at room temperature. These were examined daily for three days, and those that contained cercariae on examination by the naked eye and the hand lens, were sorted out for further examination. This process of snail collection was continued during the months of July, August and September.

The cercariae that were found in the test-tubes containing *Bulinus* and *Planorbis* were examined under the microscope, and those that possessed a forked tail, but no pharynx and no eye spots, were

considered to be schistosome larvae. The shells of the snails containing these schistosome cercariae were broken, and their bodies with the infected livers dissected out, and immediately preserved in 10 per cent. formalin or Bouin. They were then passed through changes of alcohol, xylol, and embedded in paraffin. Serial sections were cut from these, stained with haematoxylin and counterstained with eosin in the usual way, and examined serially. The critic may argue against this method of serially sectioning the cercariae and counting all the glands possessing nuclei, by saying that the nucleus of any one gland being a big one may be cut into two and this would give a false count of two glands. This fallacy is checked and guarded against in the writer's technique by laying stress on the presence of the nucleolus before counting a cell possessing a nucleus as one individual gland. The nucleolus being a small body cannot be divided (by the microtome) into two stainable and visible halves.

Besides making these sections the writer tried many methods of immediate fixation of the living cercariae, and various staining processes, but with little satisfactory result; it may be that by repeated manipulations and variation in the technique and time of these staining methods one can get good results. It was found that by preserving the infected livers and sectioning them serially and staining with haematoxylin and eosin, very satisfactory and convincing preparations were obtained.

One thing which is very noticeable in the sections of the cercariae of both *S. haematobium* and *S. mansoni* is the presence of two distinct varieties of secretory gland in any one cercaria. These are:—

1. Large flask-shaped, anteriorly-placed glands situated just in front of the ventral sucker with coarse granules which take the eosin stain intensely, i.e., oxyphilic.

2. The other glands are placed posteriorly to the large set, are smaller in size, and their protoplasm is finely granular, the granules taking the haematoxylin stain, i.e., basophilic. Both varieties of gland possess a distinct round and spongy nucleus with a nucleolus in the centre.

The sections in the secretory glands of many cercariae were easily followed in the serial sections, and were observed in many planes in the different cercariae. Thus in some, the sections were cut transversely, in others longitudinally, and still in others obliquely;

in the transverse sections one could see either the anterior coarsely granular or the posterior finely granular glands according to the level of the section ; in the longitudinal sections one could see some of the anterior oxyphilic and some of the posterior basophilic glands in one section.

CERCARIAE FROM *BULINUS CONTORTUS* AND *B. DYBOWSKI*

The secretory glands were counted in the serial sections of many single cercariae, and the writer was able to count in many of these single cercariae of *S. haematobium* up to ten glands or five pairs :—

1. Two pairs situated anteriorly, of large size each with a large circular and spongy nucleus containing a distinct large nucleolus ; the protoplasm of these glands is very coarsely granular, the granules take the eosin stain rather intensely, i.e., oxyphilic. In many of the cells these coarse granules were scanty in amount specially in the centre around the nucleus, and the latter, thus losing its external support, is noticed to be shrunken, so that the nuclear membrane, the spongy reticulum, and the nucleolus appear as one solid mass. The scantiness of the granules in these glands may have been due to excessive secretion from these during life, or the granules may have dissolved or fallen out in the processes of embedding, sectioning and staining. The nucleus in such glands, instead of appearing round as described above, appear smaller, irregular in outline, and forming one solid mass with the nucleolus.

2. Behind these anteriorly placed glands are three pairs of posteriorly placed glands which are smaller in size and contain a distinct round nucleus with a central nucleolus. The protoplasm is finely granular, the granules taking the haematoxylin stain, i.e., basophilic.

No glands other than the above mentioned five pairs were found. This conclusion is borne out by the accompanying figures. The writer's results thus differ widely from those of Manson-Bahr and Fairley on the secretory glands of the cercaria of *S. haematobium* in Egypt, both in the number and morphological appearance of these glands. On the other hand the writer's findings agree with those of Blacklock and Thompson with regard to the number and morphology of the secretory glands of the cercaria of *S. haematobium*.

Rats, which were exposed to infection from these snails, on

subsequent post-mortem examination two months later, failed to show infection with the adult worms. This may be due to the small number of cercariae that were present in the infecting dose, as they were taken from single snails and they did not count more than thirty to forty cercariae.

CERCARIAE FROM *PLANORBIS BOISSYI*

With regard to the number of the secretory glands in the cercaria of *S. mansoni* the writer found that they are six pairs, which are of two varieties:—

1. Two large flask-shaped, anteriorly-placed pairs of glands situated just in front of the ventral sucker with coarse granules in their protoplasm which take the eosin stain intensely, i.e., acidophilic.
2. Four small pairs of secretory glands situated posterior to the anterior acidophilic glands, and their protoplasm is finely granular, the granules taking the haematoxylin stain, i.e., basophilic.

Both varieties of gland possess a distinct round and spongy nucleus with a large nucleolus in the centre.

The anterior oxyphilic coarsely granular, and the posterior basophilic finely granular glands of the cercaria of *S. mansoni*, are very similar in morphology and staining reactions to the corresponding anterior and posterior glands of *S. haematobium*.

The reader can thus see that these results differ from those of Khalil and are in full agreement with the findings of Faust on the secretory glands of the cercaria of *S. mansoni* with regard to their number, morphology and staining reactions. This conclusion is borne out by the accompanying figures. Two (Nos. IV and V) of the figures are made from sections of the liver of a *Planorbis boissyi*, which proved to harbour cercariae conforming with the description given above for the schistosome cercariae, i.e., bifid tail, absence of eye spots and no pharynx. A rat was infected by the cercariae discharged from this snail, and after about two months was killed, and a large number of male immature *S. mansoni* worms were collected from its liver, so that the identity of the cercaria from the snail as that of *S. mansoni* was beyond doubt. Other rats, infected with cercariae conforming in morphology to schistosome larvae and collected from single *Planorbis boissyi* snails, did not harbour infection with adult worms on subsequent post-mortem examination. This negative result may be due to the fact that the number of infecting

cercariae was small; they were taken from single snails and were poured into a flat dish about 10 inches in diameter, containing about half a litre of tap water; in this the rat was kept standing with its feet and tail immersed for about half to one hour. Moreover the movement of the cercariae in the test tubes immediately before the infection of the rats was noticed in many cases to be sluggish, and this may also account for the failure of infection.

From the above findings the writer agrees with Faust that it is possible, at least in the case of the cercariae of *S. haematobium* and *S. mansoni*, to differentiate these two species from each other on the basis of the number of the secretory glands.

It may not be out of place to say a few words about the rat that gave a positive infection with immature male *Schistosoma mansoni* worms, after infecting it with the cercariae from one *Planorbis boissyi* snail. The worms recovered from its liver were all immature males. Professor Khalil Bey informed me that he obtained similar results by infecting animals with the cercariae from single snails, i.e., he got only one sex infection. This observation leads us to believe that a patient, in order to pass schistosome ova in his urine and faeces, or to have these deposited in his organs, may have to be infected by cercariae from more than one snail, so that some may develop into males and others into females, and after reaching maturity and mating, the females begin to pass their ova. It also may explain another observation which the writer has occasionally made in performing post-mortems on some of the bilharzial patients. It is the routine in performing post-mortems in the Kasr-el-Aini Hospital to open the portal vein, scoop out the blood and examine it for schistosomes. In some few cases the writer has observed that the portal blood harboured many worms, while the bladder and intestine showed no naked eye or microscopic evidence of schistosome infection. This finding was attributed to the possibility that the patient was recently infected, and the worms in the portal vein were still immature. In the light of the above observation, however, it seems probable that such patients may be harbouring a one-sex infection. It was not the routine to identify the sex of the worms that were recovered from these cases from the portal vein.

In conclusion the writer has to thank Professor Khalil Bey, director of the Parasitology Department, Egyptian University, and his assistants, for extending facilities in his laboratory.

DESCRIPTION OF FIGURES.

FIG. I (Sections 1 and 2).

Shows two longitudinal serial sections involving the whole region of the secretory glands in an *S. haematobium* cercaria. The sections before and after these contained no glands. Each of the two sections contains three finely granular basophilic glands on the left, and two coarsely granular acidophilic glands to the right of each section, making five pairs in all. There is a large rounded nucleus with a distinct nucleolus in each cell.

FIG. II (Sections 1, 2 and 3).

Shows three transverse serial sections in an *S. haematobium* cercaria. Section 1 shows three coarsely granular acidophilic glands; Section 2 shows one coarsely granular acidophilic and three finely granular basophilic glands; Section 3 shows three finely granular basophilic glands; making in the three sections five pairs of glands. Each gland has a rounded nucleus with a distinct nucleolus.

FIG. III (Sections 1 and 2).

Shows two longitudinal serial sections in an *S. haematobium* cercaria. Each section consists of two coarsely granular acidophilic and three finely granular basophilic glands, each of which have a nucleus and a nucleolus.

FIG. IV (Sections 1, 2, 3 and 4).

Shows four oblique serial sections in an *S. mansoni* cercaria. Section 1 shows one coarsely granular acidophilic gland; Section 2 shows two coarsely granular acidophilic and three finely granular basophilic glands; Section 3 shows one coarsely granular acidophilic and three finely granular basophilic glands; and Section 4 shows two finely granular basophilic glands; making in all four sections six pairs of glands. Each gland possesses a rounded nucleus and a nucleolus.

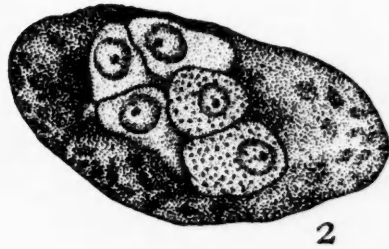
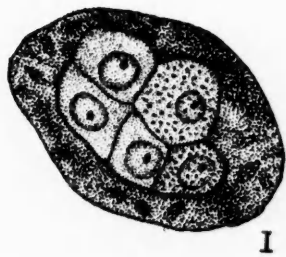


FIG. I



FIG. II

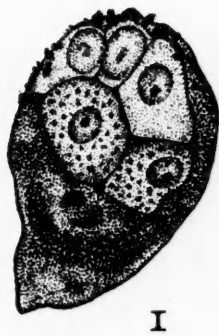


FIG. III

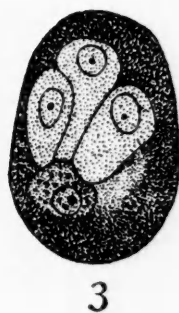
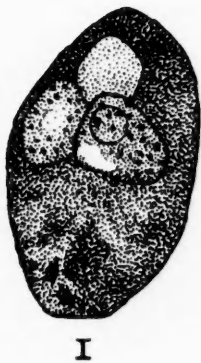


FIG. IV

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DESCRIPTION OF FIGURES.

FIG. V (Sections 1a, 2a, 3a, 4a, 5a and 6a).

Shows six transverse serial sections in an *S. mansoni* cercaria.

- Section 1a. Shows one finely granular cell possessing a nucleus and a nucleolus.
 „ 2a. Shows one finely granular cell possessing a nucleus without nucleolus.
 „ 3a. Shows four finely granular cells possessing each a nucleus and a nucleolus.
 „ 4a. Shows three finely granular cells possessing each a nucleus and a nucleolus.
 „ 5a. Shows two coarsely granular acidophilic cells each with a nucleus and a nucleolus.
 „ 6a. Shows two coarsely granular acidophilic cells each with a nucleus and a nucleolus, and a third coarsely granular cell possessing a nucleus but no nucleolus.

By summing up we find that in Fig. V the cells that have a nucleus and a nucleolus are twelve in number: four coarsely granular acidophilic and eight finely granular basophilic glands. But there are two extra cells, one acidophilic and the other basic, having each a nucleus without a nucleolus. These will not count as individual glands but are considered to be half-cells with half-nuclei. The other half of these cells with the nucleolus and half nucleus is to be seen in the serial section immediately before or after such a cell.

N.B.—Figures IV and V are made from the cercariae in the liver of the *Planorbis boissyi* snail, the living cercariae discharged from which developed to immature *S. mansoni* male worms by passing them into a white rat.

FIG. VI (Sections 1, 2 and 3).

Shows three longitudinal serial sections in an *S. mansoni* cercaria. Sections 1 and 2 show each two coarsely granular acidophilic, and three finely granular basophilic glands, all possessing nuclei and nucleoli, except the lower most finely granular cell in section 2, which has no nucleolus. A glance will show that this cell is the completing portion to the lowest cell in section 1, and both make one individual gland. Section 3 shows three finely granular cells with their nuclei and nucleoli, so that the cercaria in Fig. VI shows a sum total of four coarsely granular and eight finely granular glands.

FIG. VII (Sections 1, 2, 3 and 4).

Shows four oblique serial sections in an *S. mansoni* cercaria.

- Section 1. Has two finely granular basic glands.
 „ 2. Has three finely granular basic glands and two coarsely granular acidophilic ones.
 „ 3. Has three finely granular basic glands and one coarsely granular acidophilic one.
 „ 4. Has one coarsely granular acidophilic gland.

The total number of the glands is twelve, four coarsely granular and eight finely granular cells each possessing a nucleus and a nucleolus.

The enlargement of all the figures is 580.

2I



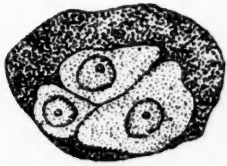
1 a



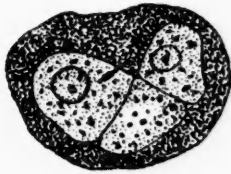
2 a



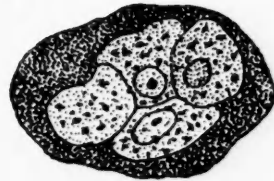
3 a



4 a

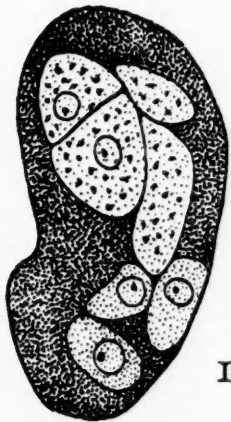


5 a

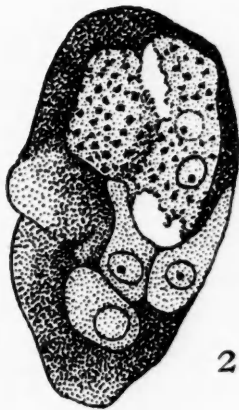


6 a

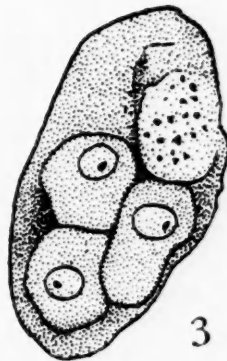
FIG. V



I

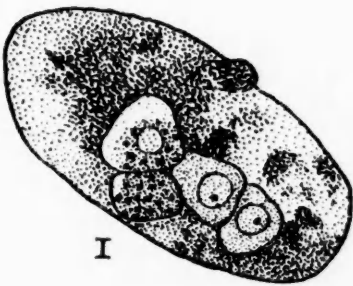


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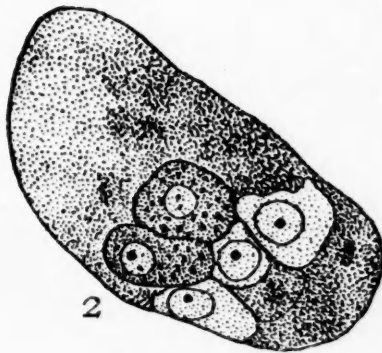


3

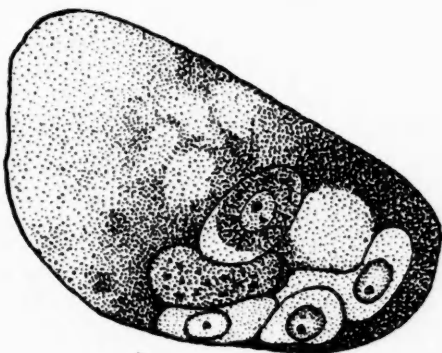
FIG. VI



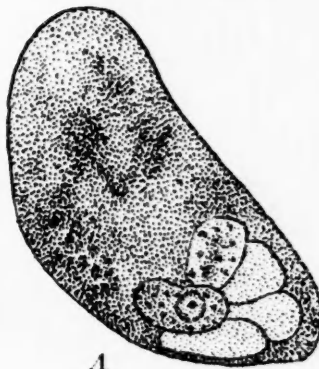
I



2



3



4

FIG. VII

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NEW ZEALAND BITING MIDGES (*DIPTERA, CERATOPOGONIDAE*)

BY

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In a former report* (1931), written in collaboration with Dr. A. Ingram, an account was given of a small collection of New Zealand *Ceratopogonidae*. Recently a larger collection of these insects has been received at the British Museum (Natural History), London, from Dr. A. Tonnoir, and, thanks to the kindness of Dr. F. W. Edwards, I have been privileged to examine this also, and to report on the species which it contains.

The collection consists of 131 specimens, almost all of which had been taken by Dr. Tonnoir. In the pages which follow, this fact is implicit, and the collector's name is given only in the cases of specimens taken by other entomologists. The specimens are referable to the following genera:—*Forcipomyia* (64), *Atrichopogon* (29), *Dasyhelea* (9), *Stilobezzia* (8), *Monohelea* (10), and *Palpomyia* (11). *Forcipomyia* predominates, almost 50 per cent. of the specimens belonging to this genus. The descriptions of three females, one *Forcipomyia* and two *Dasyhelea*, are deferred until further material is available, because, although they probably represent three new species, the single specimen of each shows no very conspicuous diagnostic features, and in the genera to which they belong the main specific distinctions are to be found in the males. It is noteworthy that there are no specimens of *Culicoides*, or the other genera known to attack human beings. It is also of considerable interest that several species resemble closely species from the faunal area of Patagonia and South Chile. This is particularly noteworthy

* *Annals of Trop. Med. & Parasitol.*, XXV, pp. 195-209.

in the case of two somewhat unusual species described here under the names *Forcipomyia* (*Apelma*) *austrina* and *Dasyhelea egregia*, which correspond with the South American species *F. (A.) limnetis* I. & M. and *D. brevipalpis* I. & M.

The terms used in the descriptions which follow have previously been defined. It may be well to make it clear, however, that measurements of radial cells, when given, are internal dimensions, that is, refer to the cells themselves, and do not include the veins enclosing them; that the length of the terminal segment of the antenna includes the stylet if present; and that asymmetrical structures (such as the harpes of some *Dasyhelea*) are figured as they appear under the microscope. The unit used is approximately 3.6μ .

Two species are named in honour of Dr. A. Tonnoir, who formed the collection, and who has contributed so largely to the scientific study of entomology in New Zealand. Others, for which it would not be easy to find descriptive names, have been called after prominent figures in the early history of the Dominion, namely, three species of *Forcipomyia* after the discoverers Tasman, Cook and de Surville, and four species of *Atrichopogon* after the first Governors, Hobson, Shortland, Fitzroy and Grey. It may be well to add that none of these species is known to be harmful to man. From the point of view of the indigenous people, indeed, they may be thought to compare favourably in this respect with one or two of the illustrious men whose names they will bear!

FORCIPOMYIA (Mg.), Kieff.

As noted in the introduction to this report, *Forcipomyia* appears to be the commonest genus of the family in New Zealand. Over 49 per cent. of the specimens in the collection belong to this genus, representing seven or eight species. A fact of some interest is that one species, *F. (Apelma) austrina* sp.n., closely resembles *F. (A.) limnetis* I. & M., a species found in the faunal area of Patagonia and South Chile. The description of one species is deferred because only a single female is available for examination. The seven species described here, or previously described, may be distinguished as shown in the key.

KEY TO THE SPECIES OF *Forcipomyia*.

1. Male without empodium; female with basal segments of antenna broader than long..... *austrina* sp.n.
Empodium in both sexes; female with basal segments of antenna less flattened 2
2. Wings adorned with pale spots 3
Wings unadorned 4
3. Wing with two large pale spots on anterior border *parvicellula* I. & M.
Wing with a single, very small, pale spot on anterior border..... *desurvillei* sp.n.
4. Lanceolate spines on tibiae; halteres white *antipodum* (Huds.)
(= *novae-zelandiae* Kieff.)
No lanceolate spines on tibiae; halteres dark brown 5
5. Femora pale in middle; harpes blade-like with dark, pointed ends *tapleyi* I. & M.
Femora dark brown in middle at least 6
6. Males (♂) 7
Females (♀) 8
7. Harpes dark brown, with broad ends tapering to a point *tasmani* sp.n.
Harpes with pale ends shaped like a hand with index finger extended *cooki* sp.n.
Harpes long and slender, with expanded, serrated ends *desurvillei* sp.n.
8. Legs dark brown; knees yellowish; without scale-like, fringed hairs *tasmani* sp.n.
Legs a duller brown; knees whitish; with scale-like, fringed hairs *cooki* sp.n.

Forcipomyia parvicellula I. & M.

Dun Mt., 1.x.1922, 1 ♀ (A. Philpott).

Forcipomyia antipodum (Huds.)

Waiho, 16.i.1922, 1 ♀; Otira, 8.ii.1922, 1 ♂ and 9.ii.1922, 1 ♀; Christchurch, 18.ii.1922, 1 ♂; Kaitouna, 19.ii.1922, 1 ♂; Kaikoura, 22.ii.1922, 1 ♀; Aniseed Valley, iii.1922, 3 ♀♀, 4 ♂♂ and 22.iii.1922, 1 ♂, 1 ♀ (mounted together on the same pin, and so presumably taken *in coitu*); Ohakune, 8.iii.1923, 2 ♂♂, 1 ♀; Nelson, 8.xi.1923, 1 ♂ and 29.xi.1923, 2 ♀♀.

F. novae-zelandiae Kieff., 1922, is probably a synonym of *F. antipodum* (Hudson 1892).

Forcipomyia tapleyi I. & M.

At the time this species was described, only the female was known. The following data regarding the male may therefore be added.

MALE. Length of wing, 2 mm. ; greatest breadth of wing, 0.5 mm.

Antennae darkish brown, plume large ; segments 4 to 11 similar, but gradually narrowing, in one specimen ranging from 16 by 14 to 16 by 11 units ; segments 12 to 15 elongate, about 80, 35, 27 and 36 by 5 to 6 units respectively ; 15 slightly broader than the others, ending in a small nipple-like process. Wings longer and narrower than in the female. First radial cell obliterated ; second small, triangular. Fork of Cu slightly distal to level of end of costa.

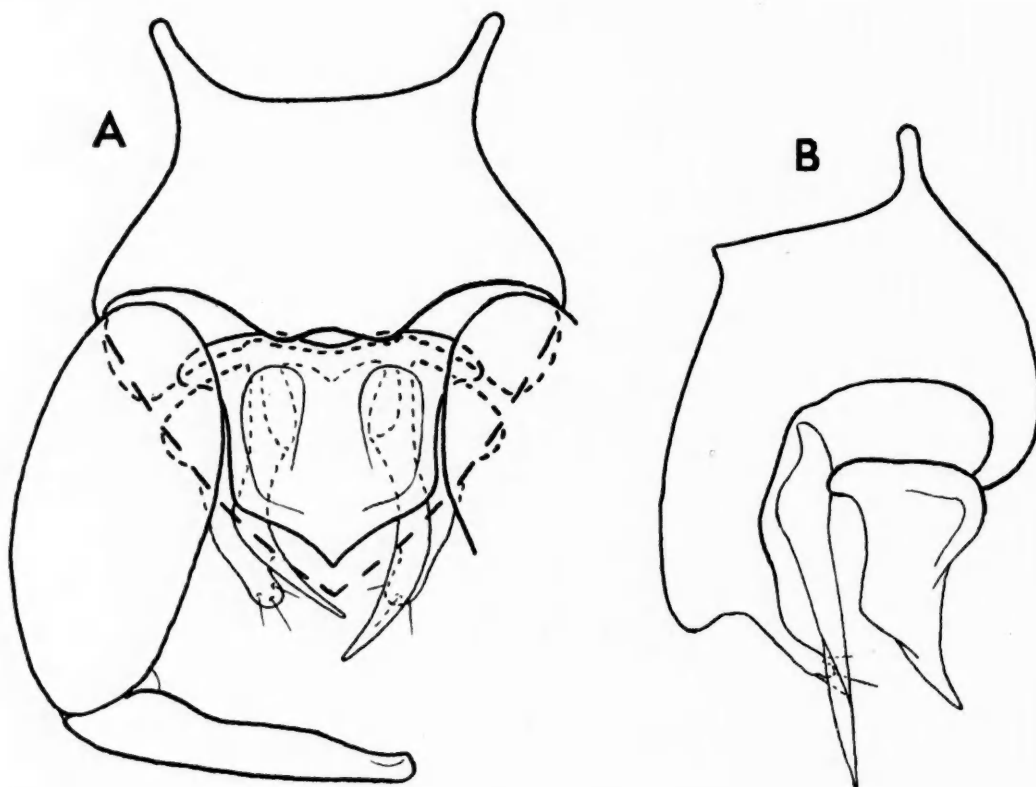


FIG. 1. *Forcipomyia tapleyi* I. & M., hypopygium. A.—ventral view ; B.—lateral view.

Hypopygium (fig. 1, A, B) very dark. Ninth sternite hairy, produced posteriorly in middle line. Side-pieces and claspers normal, very dark brown, the former bearing very long hairs. Harpes with blade-like posterior extensions which are highly chitinated at their tips, but not in their middles. Aedeagus very highly chitinated, shield-shaped.

Welton's Bush, 2.xii.1921, 1♂; Waiho, 21.i.1922, 1♂; Lake Brunner, 4.ii.1922, 3♂♂, 1♀; Christchurch, 18.ii.1922, 2♂♂; Kaitouna, 19.ii.1922, 1♀; Purau Creek, 20.ii.1922, 1♂; Kaikoura, 22.ii.1922, 1♀; Nelson, 20.iv.1922, 2♂♂.

Forcipomyia tasmani, sp.n.

This insect resembles the preceding species, *F. tapleyi*, in almost every respect. It differs chiefly in having the legs darker brown, the femora and tibiae dark brown in the middle, being usually almost entirely dark brown excepting at the knees, and for a short distance on each side of them, where they are yellowish. In the male the legs are not so dark as in the female, but the markings are similar, although paler and less extensive. The hypopygium (fig. 2, A and B) is similar to that of *F. tapleyi*, but the harpes are separate, broader and darker, and the posterior border of the aedeagus is slightly different, as shown in the figure.

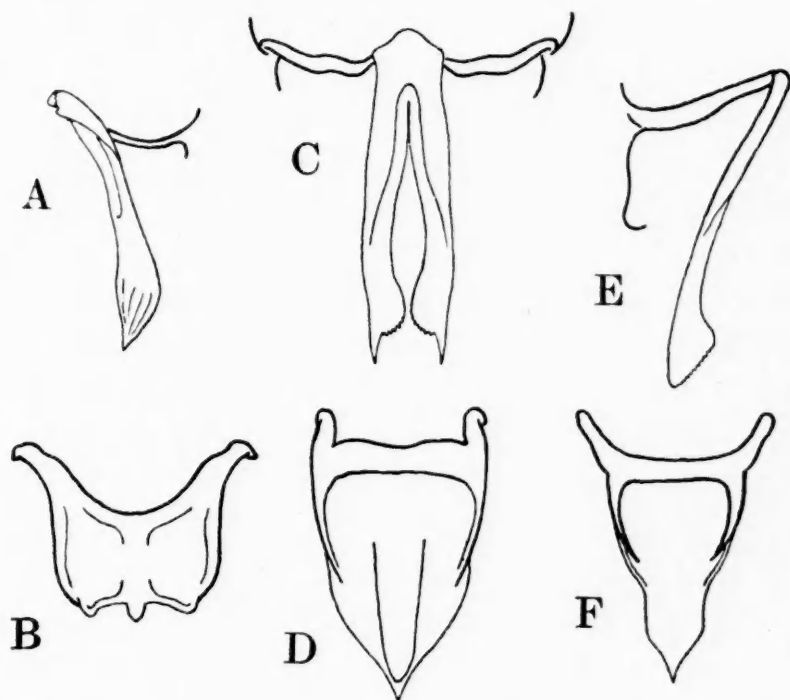


FIG. 2. Ventral views of harpes and aedeagus of *Forcipomyia tasmani*, sp.n. (A and B), *F. cooki*, sp.n. (C and D), and *F. desurvillei*, sp.n. (E and F).

Khandallah, 30.xi.1921, 2♂♂, 1♀; Wellington, 1.xii.1921, 1♀; Purau Creek, 20.ii.1922, 1♂; Kaikoura, 22 to 23.ii.1922, 4♂♂; Aniseed Valley, 2.ix.1922, 1♂ (Philpott) and 4.xii.1923, 1♂; Nelson, 14.xi.1923, 1♀ and 28.xi.1923, 1♂, 1♀; Lake Rotoroa, 13 to 20.i.1928, 2♂♂.

Forcipomyia cooki, sp.n.

This insect is almost indistinguishable from the preceding species, *F. tasmani*, but the legs are a duller brown colour, the knees whitish rather than yellowish, the T.R. slightly greater, about 0.9, and the legs more densely clothed with hairs, many of which, especially on the terminal segments of the tarsi, are scale-like and fringed. The hypopygium (fig. 2, C and D) is also very similar to that of *F. tapleyi*, but the distal extremities of the harpes are pale, yellowish, and are shaped rather like a hand with the index finger extended. The association of the males and the females in this species is purely conjectural, and may be erroneous.

Days Bay, 29.xi.1921, 1♂; Khandallah, 30.xi.1921, 1♀; Kaikoura, 23.ii.1922, 1♂; Nelson, 18.x.1923, 1♀, 6.xi.1923, 1♂ and 28.xi.1923, 3♂♂.

Forcipomyia desurvillei, sp.n.

A dark brown species closely resembling the preceding species, *F. cooki*, but with a single very small pale spot on the wing, and differing also as indicated below.

MALE. Length of wing, 1.7 mm.; greatest breadth of wing, 0.5 mm.

Eyes narrowly separated above. Palpi with lengths of last three segments 25, 11 and 11 units respectively; third segment with a very small sensory pit in basal third. Antennae darkish brown: segments 4 to 11 gradually narrowing, from 14 by 13 to 14 by 8 units; segments 12 to 15 elongate, their lengths 64, 22, 20 and 26 units respectively, 15 the broadest, and ending in a small nipple-like process. Wings similar, but with a very small pale spot on anterior margin covering the end of costa. This spot is insignificant and might be overlooked, and in partially denuded specimens might be quite indistinguishable. Fork of Cu a little distal to level of end of costa. Halteres dark brown. Legs almost uniformly dark brown, darker than in *F. cooki*, but with small pale areas at knees. Scale-like fringed hairs apparently absent. T.R. 0.9. Hypopygium (fig. 2, E and F) very dark brown, similar to that of *F. tapleyi*, but with harpes and aedeagus differing as shown in the figures, the former being long, highly chitinised rods with somewhat expanded and serrated ends.

Dun Mt., 3,000 ft., 5 to 7.i.1922, 1♂.

This insect must be distinguished from the Australian species *F. albopunctata* (Skuse), and the New Guinea species *F. punctum-album* (Kieff.), which also have the wings adorned with a single pale spot. It is darker in colour than either of these, the legs and the plume being dark brown, whereas in *F. albopunctata* the legs are pale brown and the plume golden yellow, and in *F. punctum-album* the legs are yellowish-white. It resembles in several respects the South American species *F. chilensis* (Philippi), but the harpes are entirely different.

Forcipomyia (Apelma) austrina, sp.n.

A very dark brown species, with paler brown legs; allied to *F. (A.) limnetis* I. & M., but differing as indicated below.

MALE AND FEMALE. Length of wing in both sexes about 1.3 mm.; greatest breadth of wing about 0.38 mm. in male, and 0.5 mm. in female.

Head very dark brown. Eyes bare, contiguous above. Palpi dark brown; third segment not much inflated, with a shallow pit in female, without a pit in male; lengths of last three segments about 12, 7 and 8 units respectively in male, 13, 6 and 4 units in female. Antennae dark brown, not sculptured. In male: segments 4 to 11 from sub-spherical to oval, measuring from 11 by 10 to 10 by 7 units; segments 12 to 15 elongate, their lengths 31, 26, 19 and 27 units respectively, 15 the broadest, tapering distally, ending in a short process. In female: segments 4 to 10 disc-shaped, from 5 by 9 to 6 by 8 units; segments 11 to 15 elongate, 11 to 14 sub-equal, about 15 to 17 by 7 units, 15 rather longer, about 22 by 7 units, ending in a short process. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 approximately 88, 37 and 43 units respectively.

Thorax very dark brown. Scutellum dark brown, very little paler than scutum; bearing numerous (about 30) bristles and hairs. Postscutellum very dark brown.

Wings unadorned, well clothed with dark macrotrichia, especially in female; without flat scales. Fringe long. Alula with fringe. Venation normal. Costa extending a little beyond middle of wing, 0.55 in the male, 0.6 in the female. First radial cell almost obliterated; second well formed, and rather long in the female. Petiole of median vein about as long as the very oblique cross-vein.

Fork of Cu at about level of base of second radial cell in female, of end of costa in male. Halteres with white knobs.

Legs uniformly dull yellowish-brown, paler than body; rather densely clothed with hairs, many of which, especially on the distal segments of tarsi, are somewhat scale-like, fringed, and lanceolate. Segments normally formed, without spinous armatures and without flat scales, the fourth tarsal segment about as long as fifth. T.R. in both sexes about 2. Claws small, equal; slender in male, stouter in female, with forked ends in both sexes. Empodium normal in female, absent in male.

Abdomen very dark brown. Spermathecae two, highly chitinated, sub-spherical but with a basal conical portion tapering towards the duct, sub-equal, total length about 70μ and greatest breadth about 55μ . Hypopygium very similar to, if not identical with, that of *F. (A.) limnetis*.

Reefton, 13.i.1922, 1♂; Aniseed Valley, 21.iii.1922, 1♂ and 1♀ (the two latter specimens were mounted together on the same pin and are assumed therefore to have been taken *in coitu*).

This insect is clearly related to *F. (A.) limnetis* I. & M., a South American species of which only the male is known, and may be regarded as its New Zealand counterpart since the genitalia of the males are practically identical. It differs, however, in several notable respects, for example, in having the second radial cell well developed, and the T.R. greater, and is therefore regarded as a separate, but closely allied, species.

ATRICHOPOGON, Kieff.

The collection includes 29 specimens of *Atrichopogon*, 8 males and 21 females, all almost black, with the greater part of the wings of the females clothed with macrotrichia, and closely resembling *A. vestitipennis*. The specimens had been taken in eleven different localities, and probably represent several distinct species. Clear points of distinction are, however, somewhat difficult to define, and unfortunately the structures of the hypopygium are of little assistance. The following grouping is suggested with reserve, because it is clear that if characters such as the adornment of the wings and the banding of the legs are variable, as they appear to be, others may be also.

KEY TO THE SPECIES OF *Atrichopogon*. FEMALES

1. Petiole of median vein shorter than cross-vein *vestitipennis* Kieff.
 Petiole of median vein about as long as cross-vein 2
2. Macrotrichia very few between M and Cu in the area
 between the level of the cross-vein and the base, and
 arranged in one or two rows only *hobsoni* sp.n.
 Macrotrichia numerous in this area *shortlandi* sp.n.
 No macrotrichia in this area 3
3. Large species (wing-length 2.1 mm.), almost black, with
 highly chitinated spermathecae *greyi* sp.n.
 Smaller species (wing-length 1.4 to 1.5 mm.), not so dark,
 without visible spermathecae *fitzroyi* sp.n.

Atrichopogon vestitipennis Kieff.

In a previous paper on New Zealand Ceratopogonidae, written in collaboration with Dr. A. Ingram, a description was given of a species of *Atrichopogon*, taken at Ohakune, which was provisionally identified as *A. vestitipennis* Kieff. In this description (*Annals Trop. Med. & Parasitol.*, XXV, p. 201), owing to a clerical error in the rough manuscript which was unfortunately overlooked in the proof, it is stated that there are three spermathecae, whereas actually there are only two, and the length of the chitinated portion of the duct of the spermathecae is given as about 30μ , which is rather too great and should have been about 20μ (fig. 4, A). It should also have been noted that this portion of the duct is somewhat curved. Moreover, the eyes are not entirely bare, but are sparsely hairy in part at least. In order to facilitate the differentiation of this species from others closely resembling it, which are described below, the following further particulars must be given. Regarding the distribution of macrotrichia on the wings of the female, in cell R_5 the macrotrichia extend towards the base in diminishing numbers as far as the level of the distal end of the first radial cell, and beyond the cross-vein towards the base, between M and Cu, they extend for some distance in two imperfect lines. The wings are slightly smoky, especially along the anterior border, and may show traces of two infuscated patches, the one covering the first radial cell, and the other situated between the end of the costa and the fork of the intercalary vein in cell R_5 . The costa ends almost at the level of the tip of Cu_1 , or very slightly beyond it, in the female; a little beyond this level in the male. Cu and Cu_1 practically in line, Cu_2 oblique, forming with Cu_1 an

angle of about 45° or rather more. Intercalary vein with stem usually distinct.

In the collection which forms the subject of this report, the following may be referred to this species:—

Mt. Arthur, 4,500 ft., xii.1921, 1 ♂, 4 ♀♀. These specimens are darker than those from Ohakune, the scutellum being almost black like the scutum, the legs dark or darkish brown, and the halteres varying in colour from white to dark brown (♂). The hypopygium of the single male does not show clearly the darkened antero-posterior band in the middle of the aedeagus.

Atrichopogon hobsoni, sp.n.

An almost black species resembling *A. vestitipennis* in most characters, but with the petiole of the median vein about as long as the cross-vein, and differing also as indicated below.

MALE AND FEMALE. Length of wing, 1.6 to 2.1 mm.; greatest breadth of wing about 0.5 to 0.8 mm.

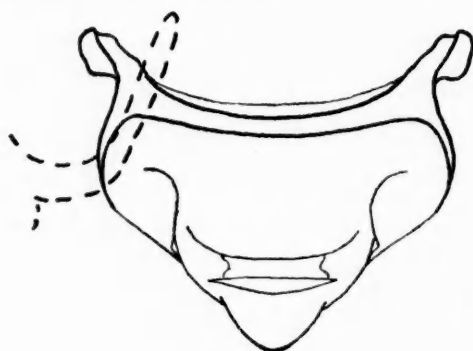


FIG. 3. *Atrichopogon hobsoni*, sp.n., aedeagus, ventral view.

Head similar. Palpi with third segment shorter than fourth and fifth together; lengths of last three segments in both sexes (small specimens) about 16, 12 and 9 units respectively. Antennae similar. In male, segments 4 to 11 measuring in one specimen from 14 by 12 to 11 by 9 units, and lengths of segments 12 to 15 in same specimen about 25, 44, 33 and 41 units respectively. In female, segments 4 to 10 sub-equal, about 9 by 9 units in one specimen, and lengths of segments 11 to 15 in same specimen about 23, 27, 28, 27 and 35 units respectively.

Thorax almost black. Scutellum in male about as dark as scutum, in female slightly paler; bearing four bristles and a few small hairs.

Wings somewhat smoky, the infuscation in some specimens (notably in those taken at Nihotapu) deepening into two dark patches on the anterior border, the one covering the first radial cell, and the other situated between the end of the costa and the fork of the intercalary vein. These dark markings are not always present, and when they are present are much less pronounced in the males than in the females. Distribution of macrotrichia similar, those between M and Cu extending towards base beyond level of cross-vein very few and arranged in one or two lines only. The petiole of median vein about as long as cross-vein. Halteres with white or sometimes brownish knobs.

Legs almost uniformly brown, but in some specimens showing traces of darker median bands on femora and tibiae. T.R. about 2.3 to 2.6.

Abdomen blackish. Spermathecae (fig. 4, B) two, highly chitinised oval or pyriform, tapering towards duct, sub-equal, total length about 96 to 104 μ (of which 10 to 15 μ may be regarded as the chitinised commencement of the duct), and greatest breadth about 63 to 67 μ . Hypopygium (fig. 3) similar to that of *A. vestitipennis*, but without the median antero-posterior dark band on its ventral aspect.

Nihotapu, ii.1923, 1 ♂, 2 ♀♀; Weltons Bush, 2.xii.1921 1 ♂; Waiho, i.1922, 1 ♂, 2 ♀♀; and (?) Mt. Arthur, 4,500 ft., 20.xii.1921, 1 ♀

Atrichopogon shortlandi, sp.n.

An almost black species resembling *A. vestitipennis*, but with the petiole of the median vein about as long as the cross-vein, and the wings more densely clothed with macrotrichia. Differing also as indicated below.

MALE AND FEMALE. Length of wing, 1.8 to 2.0 mm.; greatest breadth of wing, 0.5 to 0.7 mm.

Head similar. Antenna of male with twelfth segment relatively longer, the lengths of last five segments measuring in one specimen about 11, 41, 35, 30 and 29 units respectively.

Thorax similar. Scutellum in male almost black, and in female slightly, but sometimes only very slightly, paler than scutum. In male the usual paler sub-lateral antero-posterior stripes, and clear areas at each side immediately anterior to scutellum, are conspicuous.

Wings more densely clothed with macrotrichia than in *A. vestitipennis*. In female, the macrotrichia in cell R_5 extend almost to cross-vein, and between M and Cu reach almost to base, being about five or six rows deep at level of cross-vein. In male, macrotrichia are numerous in cells R_5 and M_2 , and a few are usually present also in cell M_2 . Petiole of median vein about as long as cross-vein. Halteres whitish or brownish, usually more infuscated in male than in female, and in one male (Mt. Arthur) almost black.

Legs brown or darkish brown, usually darker in males than in females; femora and tibiae darker in the middle than at their ends (and in male femora dark almost or quite to base), producing a banded appearance which, however, is not always very distinct. T.R. about 2.5.

Abdomen blackish. Spermathecae (fig. 4, c) two, highly chitinised, oval, sub-equal, measuring in one specimen about 105μ by 75μ ; the chitinised portion of duct straight and short, about 10 to 15μ . Hypopygium of male very similar to that of *A. vestitipennis*, if not indistinguishable from it.

Aniseed Valley, 1 to 4.xii.1923 1♂ and 21.iii.1922, 1♀; Waiho, 30.i.1922, 1♀ (abdomen missing); Lake Brunner, 4.ii.1922, 1♀; Kaitouna, 19.ii.1922, 1♀; Kaikoura, 22.ii.1922, 2♂♂, 3♀♀; Maitai Valley, iii.1922, 2♀♀ (one without abdomen); and (?) Mt. Arthur, 4,500 ft., 20.xii.1921, 1♂.

Atrichopogon fitzroyi, sp.n.

A dark brown species resembling in general characters *A. vestitipennis*, but with the petiole of the median vein about as long as the cross-vein, and the wings less densely clothed with macrotrichia. Differing also as indicated below.

FEMALE. Length of wing, 1.4 to 1.5 mm.; greatest breadth of wing, 0.5 mm.

Head similar. Eyes almost entirely bare. Palpi similar. Antennae similar, but rather paler brown; lengths of last six segments measuring in one specimen about 9, 23, 23, 24, 24 and 32 units respectively, and their breadths (when not compressed) about equal, 6 to 7 units.

Thorax similar but not so dark, and showing more clearly traces of the usual adornment. Scutellum rather paler than scutum; bearing four bristles, and about a dozen small hairs.

Wings less densely clothed with macrotrichia, those in cell R_5 extending basally only to about middle of second radial cell, and those between M and Cu not extending beyond level of cross-vein. Petiole of median vein about as long as cross-vein. Halteres with whitish or brownish knobs.

Legs brown, and more or less banded, the knees, basal thirds of femora and tibiae, and apical fifth of femora being paler than the rest. T.R. about 2.2.

Abdomen not so dark. Spermathecae absent or indistinguishable.

Nelson, 27.xi.1923, 1 ♀ and 3.xii.1921, 1 ♀ (damaged).

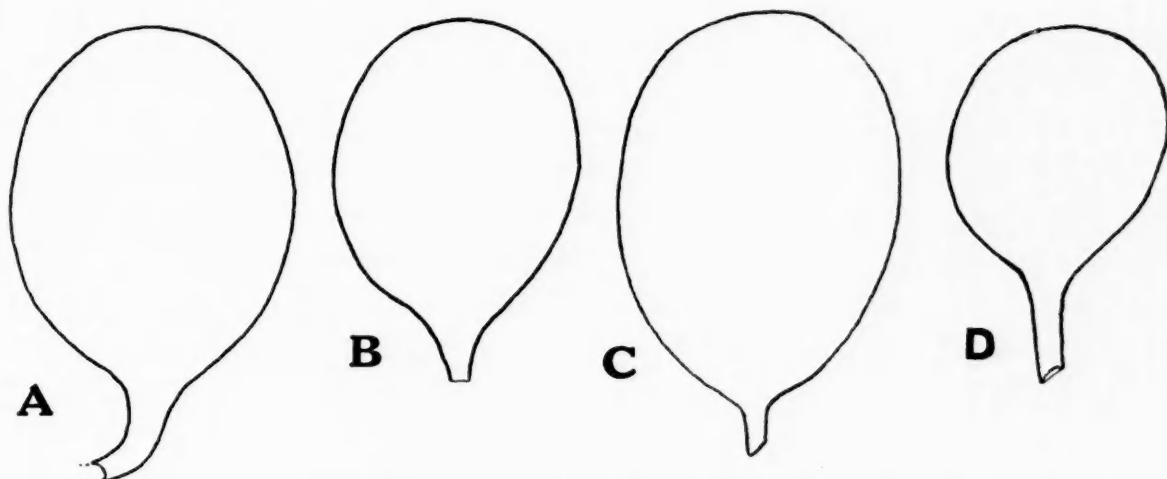


FIG. 4. Spermathecae of *Atrichopogon vestitipennis* Kieff. (A), *A. bobsoni*, sp.n. (B), *A. shortlandi*, sp.n. (C), and *A. greyi*, sp.n. (D).

Atrichopogon greyi, sp.n.

Allied to the preceding species, *A. fitzroyi*, sp.n., but larger, and darker, and differing also as indicated below.

FEMALE. Length of wing, 2.1 mm.; greatest breadth of wing, 0.8 mm.

Head similar, but darker, almost black. Antennae similar, but much darker; lengths of last six segments measuring in the unique specimen about 12, 26, 27, 31, 31 and 39 units respectively, and their breadths (uncompressed) sub-equal, about 7 units.

Thorax almost black. Scutellum slightly paler than scutum.

Wings similar, but with even fewer macrotrichia, those in cells R_5 , M_1 , and between M and Cu extending towards base only about as far as level of middle of second radial cell. Petiole of median vein about as long as cross-vein. Stem of intercalary fork not distinct. Halteres with brownish knobs.

Legs similar but darker; the lighter areas considerably narrower, femora and tibiae of hind legs almost entirely dark brown but for narrow paler zones on each side of knees. T.R. about 2.5.

Abdomen darker. Spermathecae (fig. 4, D) two, highly chitinated, oval, sub-equal, about 68μ by 55μ ; the chitinated portion of duct straight and rather long, about 25μ .

Otira, 8.ii.1922, 1 ♀.

DASYHELEA, Kieff.

The nine species of *Dasyhelea* in the collection perhaps represent five species, three of which are described below. The description of the other two is deferred, because only a single female of each is available for examination, and in this genus the main specific distinctions are usually to be found in the males. As noted in the introduction, one species is of particular interest because of its resemblance to a species found in the faunal area of Patagonia and South Chile. The species described may be distinguished as shown in the key.

KEY TO THE SPECIES OF *Dasyhelea*

1. Both radial cells obliterated *egregia* sp.n.
Second radial cell more or less distinct 2
2. Second radial cell square *jucunda* sp.n.
Second radial cell longer than broad *oribates* sp.n.

Dasyhelea egregia, sp.n.

A small, very dark brown species, with paler brown legs; allied to *D. brevipalpis* I. & M., but differing in a number of characters as indicated below.

MALE. Length of wing, 1.0 mm.; greatest breadth of wing, 0.3 mm.

Head very dark brown. Eyes widely separated above; finely but densely hairy all over. Palpi similar to those of *D. brevipalpis*;

lengths of last three segments 10, 6 and 6 units respectively. Antennae dark brown, segments not sculptured: segments 4 to 11 gradually narrowing, from 9 by 8 to 9 by 5 units; segments 12 to 15 elongate, not binodose, their lengths in one specimen about 16, 23, 12 and 13 units respectively, 15 broader than the others, tapering distally, without a stylet.

Thorax very dark brown. Humeral pits present. Scutellum about as dark brown as scutum; bearing 6 bristles, and about 15 small hairs. Postscutellum very dark brown.

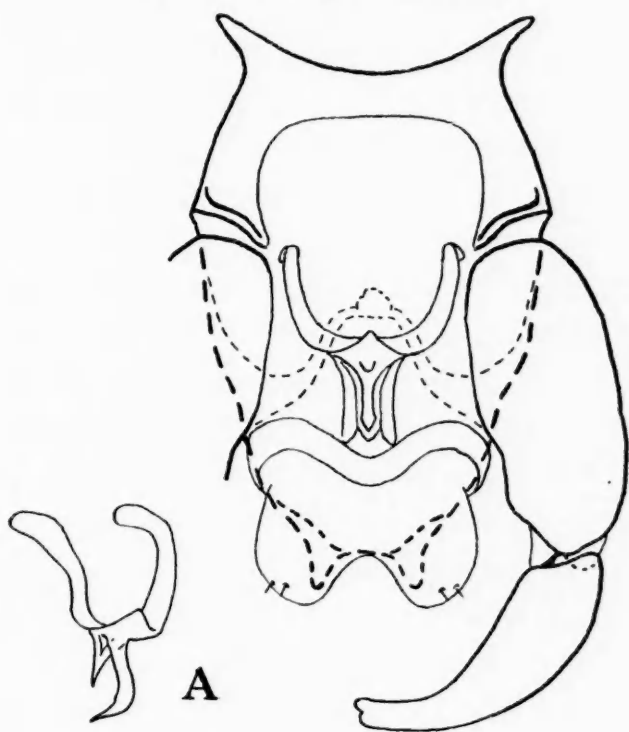


FIG. 5. *Dasybelea egregia*, sp.n., hypopygium, ventral view. A.—Oblique view of chitinised portions of aedeagus.

Wings unadorned, moderately well clothed with macrotrichia. Fringe long; alula with fringe. Venation normal, veins very pale. Costa reaching to about middle of wing. Both radial cells appear to be obliterated. M_2 deficient at its proximal end, but petiole of median vein is probably longer than the very oblique cross-vein. Fork of Cu distal to level of end of costa. Halteres with pale, brownish knobs.

Legs uniformly dull brown; segments unarmed and not inflated. T.R. about 1.7. Fourth tarsal segment cylindrical, not so long as fifth. Claws normal. Empodium minute.

Wings similar, but with even fewer macrotrichia, those in cells R_5 , M_1 , and between M and Cu extending towards base only about as far as level of middle of second radial cell. Petiole of median vein about as long as cross-vein. Stem of intercalary fork not distinct. Halteres with brownish knobs.

Legs similar but darker; the lighter areas considerably narrower, femora and tibiae of hind legs almost entirely dark brown but for narrow paler zones on each side of knees. T.R. about 2.5.

Abdomen darker. Spermathecae (fig. 4, D) two, highly chitinated, oval, sub-equal, about 68μ by 55μ ; the chitinated portion of duct straight and rather long, about 25μ .

Otira, 8.ii.1922, 1 ♀.

DASYHELEA, Kieff.

The nine species of *Dasyhelea* in the collection perhaps represent five species, three of which are described below. The description of the other two is deferred, because only a single female of each is available for examination, and in this genus the main specific distinctions are usually to be found in the males. As noted in the introduction, one species is of particular interest because of its resemblance to a species found in the faunal area of Patagonia and South Chile. The species described may be distinguished as shown in the key.

KEY TO THE SPECIES OF *Dasyhelea*

1. Both radial cells obliterated *egregia* sp.n.
 Second radial cell more or less distinct 2
2. Second radial cell square *jucunda* sp.n.
 Second radial cell longer than broad *oribates* sp.n.

Dasyhelea egregia, sp.n.

A small, very dark brown species, with paler brown legs; allied to *D. brevipalpis* I. & M., but differing in a number of characters as indicated below.

MALE. Length of wing, 1.0 mm.; greatest breadth of wing, 0.3 mm.

Head very dark brown. Eyes widely separated above; finely but densely hairy all over. Palpi similar to those of *D. brevipalpis*;

lengths of last three segments 10, 6 and 6 units respectively. Antennae dark brown, segments not sculptured: segments 4 to 11 gradually narrowing, from 9 by 8 to 9 by 5 units; segments 12 to 15 elongate, not binodose, their lengths in one specimen about 16, 23, 12 and 13 units respectively, 15 broader than the others, tapering distally, without a stylet.

Thorax very dark brown. Humeral pits present. Scutellum about as dark brown as scutum; bearing 6 bristles, and about 15 small hairs. Postscutellum very dark brown.

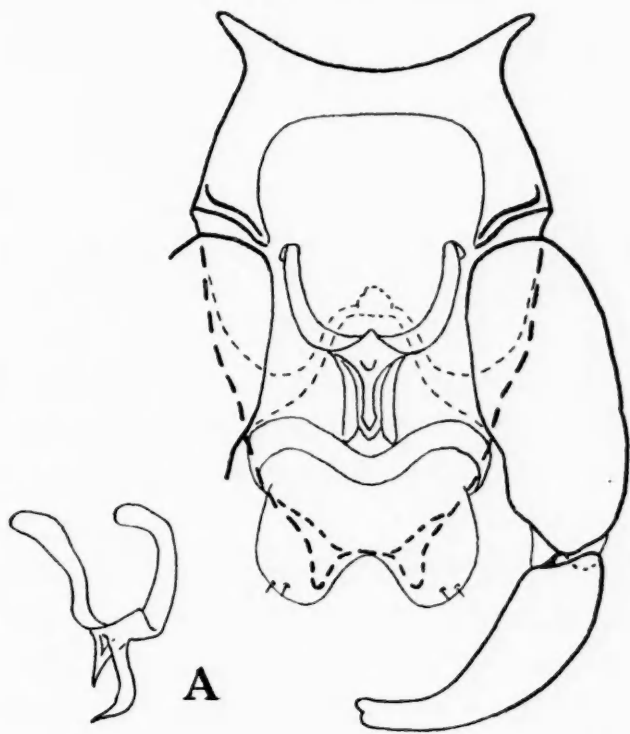


FIG. 5. *Dasybelea egregia*, sp.n., hypopygium, ventral view. A.—Oblique view of chitinised portions of aedeagus.

Wings unadorned, moderately well clothed with macrotrichia. Fringe long; alula with fringe. Venation normal, veins very pale. Costa reaching to about middle of wing. Both radial cells appear to be obliterated. M_2 deficient at its proximal end, but petiole of median vein is probably longer than the very oblique cross-vein. Fork of Cu distal to level of end of costa. Halteres with pale, brownish knobs.

Legs uniformly dull brown; segments unarmed and not inflated. T.R. about 1.7. Fourth tarsal segment cylindrical, not so long as fifth. Claws normal. Empodium minute.

Abdomen dark brown. Hypopygium (fig. 5) very similar to that of *D. brevipalpis*, but differing in detail as shown in figure.

Nelson, 25.ix.1922, 3♂♂.

This insect is clearly allied to *D. brevipalpis* I. & M., a South American species of which also only the male is known, but is smaller, darker, and differs in a number of minor characters, such as the number of bristles on the scutellum, and the T.R., as well as in the details of the structure of the hypopygium.

Dasyhelea jucunda, sp.n.

A small, almost black species, with dark brown legs, the scutellum almost as dark as the scutum, and the halteres dark brown but with a whitish substance in the knobs which may show through.

MALE AND FEMALE. Length of wing, about 1.3 mm.; greatest breadth of wing, about 0.4 mm.

Head almost black. Eyes densely hairy all over; contiguous above, the facets separated by a narrow line. Palpi dark brown, third segment without a definite pit; in male, last three segments sub-cylindrical, their lengths about 26, 11 and 15 units respectively; in female, third segment somewhat inflated at base, the lengths of last three segments about 17, 8 and 8 units respectively. Antennae dark brown, segments finely sculptured. In male, segments 4 to 11 gradually narrowing, from 10 by 13 to 12 by 8 units; segments 12 to 15 elongate, sub-equal, their actual lengths in the specimen measured being 27, 30, 29 and 25 units respectively, 12 to 14 binodose, 15 slightly broader than the others, tapering distally, without a stylet. In female, segments 4 to 10 oval, tapering distally a little, measuring in the unique specimen from 9 by 6 to 10 by 5 units; segments 11 to 14 sub-equal, about 13 by 5 units; 15 a little longer, about 19 units, tapering distally, without a stylet. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 approximately 72, 70 and 80 units respectively.

Thorax almost black. Scutellum almost as dark as scutum, but slightly paler in middle than at sides; bearing about 14 bristles and hairs. Postscutellum almost black.

Wings as usual. Costa reaching to about middle of wing. First radial cell obliterated; second small, almost square. Petiole of median vein not so long as cross-vein. Fork of Cu at about

level of end of costa (♀), or slightly distal to it (♂). Halteres dark brown, but knobs contain a whitish substance which may show through to some extent.

Legs almost uniformly very dark brown, only first 1 to 2 segments of tarsi a little paler. Form of segments and of claws normal. T.R. about 2.3.

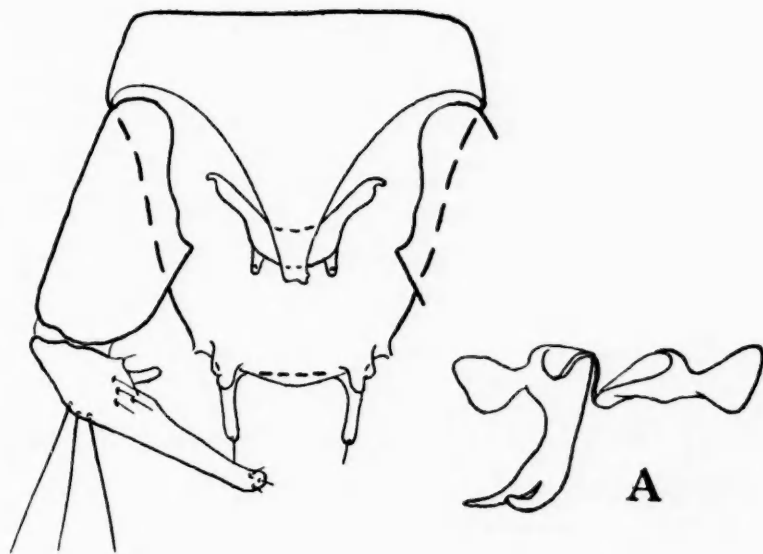


FIG. 6. *Dasyhelea jucunda*, sp.n., hypopygium, ventral view. A.—harpes.

Abdomen almost black. Spermatheca single, highly chitinated, sub-spherical, diameter about 40μ , and duct hardly at all chitinated. Hypopygium (fig. 6) very dark, highly chitinated. Ninth sternite without bristles, prolonged posteriorly in middle as a conical process projecting beyond aedeagus. Ninth tergite bearing on its posterior margin two long finger-like processes. Side-pieces with ventral side clothed with numerous bristles, but on dorsal side only a transverse sub-apical row. Claspers with a small branch near base. Harpes (fig. 6, A) forming a very asymmetrical and unevenly chitinated transverse band from the right side of which arises a large, forked posterior projection. Aedeagus of the common form.

Christchurch, 17.ii.1922, 1 ♂; and probably Kaikoura, 23.ii.1922, 1 ♂ (from which the end of the abdomen with the hypopygium is missing), and 1 ♀. The incomplete specimen referred to in a previous report (*Annals Trop. Med. & Parasitol.*, XXV, p. 202) belongs also to this species. The association of the female with the males is only conjectural, and may be erroneous. This insect resembles the

South American species *D. andensis* I. & M., but the hypopygium is distinct although somewhat similar, and differs in several respects, notably in the form of the claspers and harpes.

Dasyhelea oribates, sp.n.

Allied to the preceding species, *D. jucunda*, differing only as indicated below.

MALE. Length of wing, 1.3 mm.; greatest breadth of wing, 0.37 mm.

Eyes more widely separated above. Palpi with third segment somewhat inflated at base, but without a pit; lengths of last three segments in the unique specimen 20, 10 and 9 units respectively. Antennae with basal segments similar, measuring from 10 by 13 to 12 by 8 units; segments 12 to 15 successively shorter, their lengths 31, 29, 21 and 19 units respectively. Scutellum bearing six bristles (two lateral, four admedian) and five small hairs. Second radial cell longer than broad, more oblong, and not so clearly open as in male *D. jucunda*. Fork of Cu at the level of end of costa. Halteres

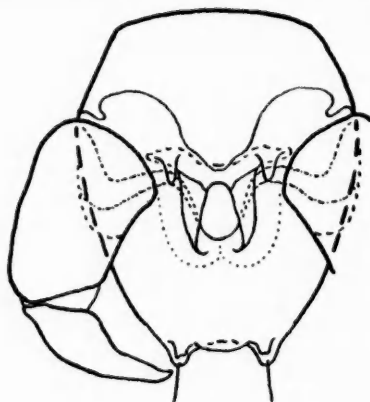


FIG. 7. *Dasyhelea oribates*, sp.n., hypopygium, ventral view.

almost black. Legs similar. T.R. 2. Hypopygium (fig. 7) distinctive. Ninth sternite without bristles, produced posteriorly in middle, and bearing at each side a highly chitinised finger-like process which at first glance appears to be part of aedeagus. Processes on posterior margin of ninth tergite, small. Side-pieces and claspers small, the latter unbranched, normal. Harpes somewhat resembling those of *D. reynoldsi* I. & M., broad and ribbon-like at sides, and joined across middle line by a very slender band. Aedeagus with a broad and highly chitinised basal bar, and arising dorsal to it a pair of rather delicate plates which taper posteriorly to sharp, curved points.

Dun Mt., 3,000 ft., 5 to 7.i.1922, 1♂.

STILOBEZZIA, Kieff.

The nine specimens of *Stilobezzia* in the collection represent three species, *S. ohakunei* I. & M., and two rather closely allied species. The New Zealand species of this genus at present known may be readily distinguished by superficial characters as shown in the key.

KEY TO THE SPECIES OF *Stilobezzia*.

1. First tarsal segment of hind legs with a basal spine; very dark brown species *antipodalis* I. & M.
First tarsal segment of hind legs without a basal spine 2
2. Yellowish-brown species, with a yellowish-brown scutellum *ohakunei* I. & M.
Darker, chestnut-brown species 3
3. Wings of male with macrotrichia fairly numerous in cells M_1 and M_2 *badia* sp.n.
Wings of male with few macrotrichia in cell M_1 , and only one or two in cell M_2 *tonnoiri* sp.n.

Stilobezzia ohakunei I. & M.

Lake Brunner, 3.ii.1922, 1♀; Nihotapu, 23.ii.1922, 1♀. The scutellum of the specimen from Nihotapu is armed with five bristles and five small hairs.

Stilobezzia badia, sp.n.

A chestnut-brown species, with the scutellum orange or yellowish, paler than the scutum, the halteres yellowish, and the legs almost uniformly dull brown.

MALE and FEMALE. Length of wing: male, 1.9 mm.; female, 2.3 mm.; greatest breadth of wing: male, 0.55 mm.; female, 0.76 mm.

Head darkish brown. Eyes bare, separated above by a narrow space. Mandibles of female armed with seven strong teeth. Palpi darkish brown, segments sub-cylindrical; lengths of last three segments in the unique female, 21, 11 and 20 units respectively, and third segment with a shallow sub-apical depression bearing sensory hairs. Antennae in male darkish brown; segments 4 to 12 similar, oval, measuring from 14 by 10 to 12 by 7 units, the last three being sub-equal; segments 13 to 15 elongate, their lengths 51, 49 and 47 units respectively, and their breadths from 5 to 7 units, 15 the broadest, not dilated at base, without a stylet. In female (fig. 8, c), almost uniformly dark brown, segments sub-cylindrical; segments 4 to 10 measuring from 16 by 7 to 20 by 6 units; segments 11 to 14

sub-equal, about 35 to 38 by 5 to 6 units ; 15 rather longer, 42 by 6 units, without a stylet. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 measuring 190, 125 and 154 units respectively.

Thorax almost uniformly dull chestnut-brown, the adornment as usual but indistinct, and shoulders hardly at all paler. Tuft above wing-base composed of about eight hairs. Scutellum paler than scutum, orange-yellow in female, paler brown in male ; bearing 6 to 7 bristles, and about six small hairs. Postscutellum dark brown.

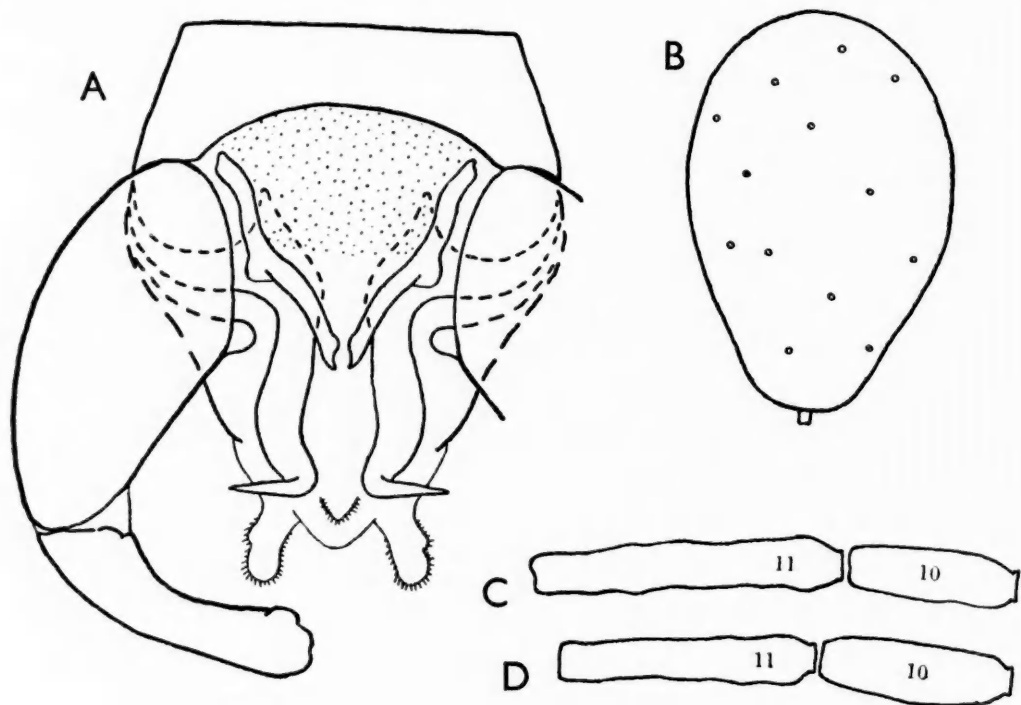


FIG. 8. *Stilobezzia badia*, sp.n. A.—hypopygium, ventral view; B.—spermatheca; C.—segments 10 and 11 of antenna of female. D.—segments 10 and 11 of antenna of female *S. obakunei* I. & M.

Wings brownish, unadorned, of usual form. Macrotrichia abundant on distal third, numerous in cells R_5 , M_1 and M_2 , and in female a few also between rami of Cu, and one or two in anal cell. Costa extending about four-fifths of wing-length. First radial cell rhomboidal, about four times as long as broad in female, but rather narrower in male ; second large, about three times as long as first. Cross-vein and distal part of R_1 not in line. Petiole of median vein slightly longer than cross-vein. Fork of Cu almost at level of cross-vein in female, distal to it in male. Halteres with pale, yellowish-brown knobs.

Legs almost uniformly dull brown, knees a little darker, and

terminal segments of tarsi more or less infuscated. Segments normal, not swollen, unarmed. T.R. about 2.2. First tarsal segment of hind legs without a basal spine. Claws normal.

Abdomen dark brown. Spermathecae (fig. 8, B) two (and a rudimentary third), highly chitinised, sparsely pitted, pear-shaped, unequal in the single female examined, about 82μ by 60μ and 100μ by 67μ respectively; the duct very narrow, and chitinised for only a very short distance. Hypopygium (fig. 8, A) dark brown. Ninth sternite without bristles, widely excavated in middle line posteriorly. Side-pieces normal; claspers covered with longitudinal rows of microtrichia, very dark brown, with blunt, club-like ends. Harpes highly chitinised, with broad, bifid bases, and ends which taper to points bent ventrally over the aedeagus. Aedeagus of type found in *S. ochracea* and several other species; membrane joining it to ninth sternite spiculate.

Aniseed Valley, 1 to 4.xii.1923, 1 ♀; Nelson, 28.xi.1923, 1 ♂.

This is a darker brown insect than *S. ohakunei* I. & M., of which at present only the female is known, and differs from that species also in the greater length of the terminal segments of its antenna, the number of the bristles on its scutellum, and the form of its spermathecae. The association of this male with this female is purely conjectural, and may be erroneous.

Stilobezzia tonnoiri, sp.n.

Closely allied to the preceding species, *S. badia*, differing as indicated below.

MALE. Length of wing, 1.4 to 1.7 mm.; greatest breadth of wing, about 0.55 mm.

Palpi with last three segments measuring in one specimen 17, 8 and 15 units respectively. Antennae with segments 4 to 11 similar, oval, in one specimen measuring from 16 by 8 to 15 by 7 units; 12 slightly produced distally, 19 by 7 units; 13 to 15 elongate, 14 longer than 13, measuring 36, 48 and 47 by 5 to 6 units respectively. Thorax with shoulders distinctly paler than scutum. Scutellum pale brown, paler than scutum; bearing four bristles, and one or two small hairs. Wings similar. Macrotrichia fewer; only a few in cell M_1 and one or two in cell M_2 . First radial cell rhomboidal, about five times as long as broad; second about three times as

long as first. Halteres with brownish knobs. T.R. almost 2. Hypopygium (fig. 9) not so dark. Claspers not so dark, almost normal in shape. Harpes less highly chitinised, their distal parts long, tapering, more or less curved ventrally near their extremities. Aedeagus darker, broader, differently shaped as shown in figure.

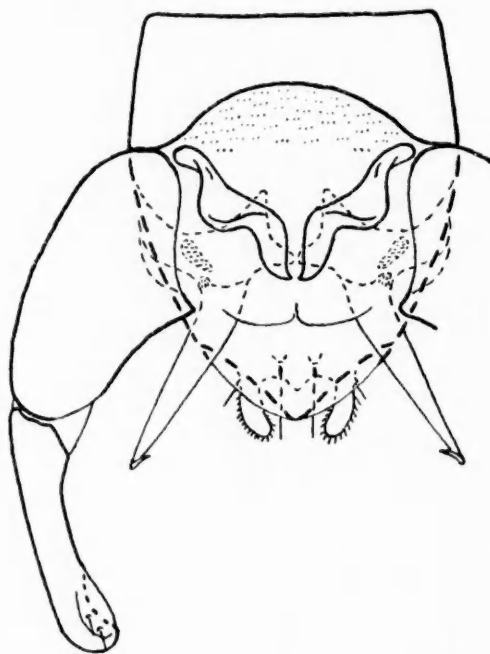


FIG. 9. *Stilobezzia tonnoiri*, sp.n., hypopygium, ventral view.

Reefton, 13.I.1922, 2 ♂♂; Nelson, 28.xi.1923, 1 ♂; Aniseed Valley, 1 to 4.xii.1923, 1 ♂.

MONOHELEA, Kieff.

The ten specimens of *Monohalea* in the collection represent four species. These, with *M. antipodalis* I. & M., can readily be distinguished by superficial characters as shown in the key.

KEY TO THE SPECIES OF *Monohalea*.

1. Wings adorned with dark markings *nubeculosa* sp.n.
Wings unadorned..... 2
2. Legs uniformly dark brown *tonnoiri* sp.n.
Legs with dark bands 3
3. Dark band on femur of hind leg reaching to the knee *clavipes* sp.n.
Dark band on femur of hind leg not reaching the knee 4
4. Dark band on tibia of hind leg occupying about one-third
of the segment *ferruginea* sp.n.
Dark band on tibia of hind leg occupying about one-fifth
of the segment *antipodalis* I. & M.

Monohalea nubeculosa sp. n.,

A very dark brown species, with wings adorned with blackish markings, and femora and tibiae entirely dark brown excepting narrowly on each side of the knees.

FEMALE. Length of wing, 1.7 mm.; greatest breadth of wing, 0.64 mm.

Head dark brown, with silvery pruinescence. Eyes bare, separated above by a wedge-shaped area. Palpi? Antennae darkish brown, proximal segments with basal half or third pale brown, last five segments almost entirely dark brown; segments 4 to 10 sub-cylindrical, sub-equal, measuring in the unique specimen from 11 by 6 units to 13 by 5 units; segments 11 to 15 elongate, sub-equal, about 19 by 5 units, 15 tapering at its end, with a stout terminal bristle but no stylet. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 measuring 96, 92 and 108 units respectively.

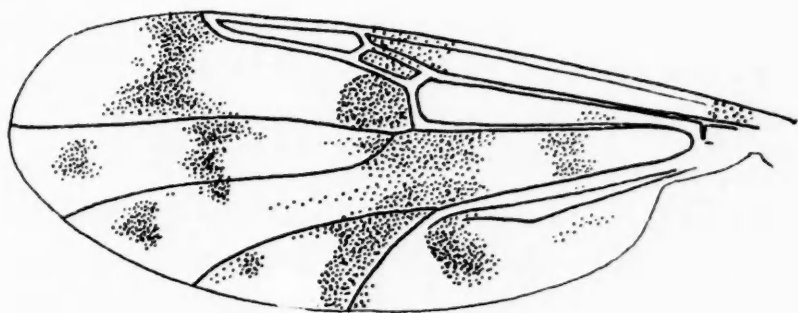


FIG. 10. *Monohalea nubeculosa*, sp.n., diagram of wing of female to show adornment.

Thorax very dark brown, with paler brown shoulders, and dorsum adorned with a silvery pruinescence much as in the West African species *M. litoraurea*; without spine or tubercle. Scutal bristles rather long, dark brown. Scutellum cream-coloured at sides, narrowly dark brown in middle and at base; bearing two admedian and two lateral bristles, and about sixteen small hairs. Postscutellum dark brown.

Wings (fig. 10) adorned with blackish markings arranged in a pattern somewhat resembling that in *M. litoraurea*. Veins pale, yellowish. Macrotrichia present at wing-tip, and extending thence in a single row along practically whole posterior margin. Fringe as usual; apparently no fringe on alula. Costa extending three-

quarters of wing-length. First radial cell large, rhomboidal, about four times as long as broad; second about twice as long as first. Petiole of median vein short, about half length of cross-vein. Fork of Cu slightly proximal to level of cross-vein. Intercalary fork not present. Anal vein bent. Halteres with cream-coloured knobs.

Legs very dark brown; the proximal tarsal segments, knees, and a narrow zone on each side of knees paler brown or yellowish. All the femora slender, unarmed. Tibiae of hind legs bearing long almost spine-like bristles as well as hairs. First tarsal segment of hind legs slightly curved at base, nearly half length of tibia, armed with a stout spine at base only. T.R. about 2. Fourth tarsal segments cylindrical or sub-cylindrical; those of hind legs about as long as fifth, and armed with a pair of sub-apical spines. Fifth tarsal segments unarmed. Claws of four anterior legs equal, barbed; those of hind legs single, apparently without any barb, about as long as fifth segment.

Abdomen darkish brown, not so dark as scutum, paler brown at apex, with a silvery pruinescence. Spermathecae not examined.

Lake Brunner, 3.ii.1922, 1 ♀.

This insect belongs to a group of species, taken in various parts of the world, which are closely allied, not easily distinguished, and in some cases possibly identical. It resembles *M. hieroglyphica* Kieff., a Peruvian species and the type of the genus, but the body is apparently darker, dark brown instead of 'jaune roux,' and the femora and tibiae are more completely dark brown. It is also closely allied to the European species *M. tessellata* (Zett.) (*illustris* Winn.), but differs in having the radial cells shorter as well as slightly in the adornment of the wings.

• *Monohalea tonnoiri*, sp.n.

An almost uniformly dark brown species, with unadorned wings, pale brown halteres, and almost uniformly dark brown legs.

FEMALE. Length of wing, 1.9 mm.; greatest breadth of wing, 0.7 mm.

Head very dark brown, with grey pruinescence. Eyes bare, separated above by a wedge-shaped area. Palpi very dark brown, segments cylindrical, the lengths of last three in one specimen 19,

13 and 19 units respectively; third not inflated, bearing sensory hairs on a depressed area on apical third. Antennae uniformly very dark brown, only the third segment paler at its base; segments 4 to 10 oval or sub-cylindrical, measuring in one specimen from 10 by 8 units to 14 by 7 units; segments 11 to 14 elongate, sub-equal, in the same specimen about 22 by 7 units; 15 rather longer and broader, about 29 by 9 units, tapering distally, with a terminal bristle, but no stylet.

Thorax very dark brown, with grey pruinescence, and paler brown shoulders. Scutal hairs sparse, long, dark. Thoracic pits conspicuous. Scutellum darkish brown, but not so dark as scutum, and paler at sides than in middle; bearing seven to eight bristles, and about twenty small hairs. Postscutellum very dark brown.

Wings much as in *M. antipodalis*, unadorned, veins yellowish. Macrotrichia at tip, in cell R_5 , and a few also in cells M_1 and M_2 . Costa extending nearly three-quarters of wing-length. First radial cell very small, triangular; second large, about three times as long as first. Petiole of median vein rather longer than cross-vein. Fork of Cu at about level of cross-vein. Halteres with pale brown knobs.

Legs almost uniformly darkish brown, darkest at knees and at apices of tibiae, but with proximal segments of tarsi rather paler. Fore legs with femora lighter than tibiae, rather stout; hind legs with femora darkest at apices, not much thickened. All femora and tibiae unarmed, but bearing long spine-like hairs. All the first tarsal segments armed with spines; those of hind legs almost straight, about one-third length of tibia, with a strong basal spine, but without spines along the shaft. T.R. nearly 2. Fourth tarsal segments sub-cylindrical, unarmed; fifth also unarmed. Claws on four anterior legs small, equal; on hind legs single and barbed (or unequal and fused basally), the one part about half, the other rather over once the length of fifth segment.

Abdomen uniformly dull dark brown. Spermathecae two (and a rudimentary third), highly chitinised, oval but tapering towards the duct, sub-equal, total length about 70μ , and greatest breadth about 40μ .

Nelson, 28.xi.1923, 6 ♀♀.

Monohalea clavipes, sp.n.

Allied to *Monohalea antipodalis* I. & M. (of which only the male is known), but with the dark brown band on the hind femora extending to the knee, and differing also as indicated below.

FEMALE. Length of wing, 2.4 mm. ; greatest breadth of wing, 0.76 mm.

Eyes widely separated above. Antennae darkish brown, proximal segments pale brown at base but with apical quarter dark brown, the last five segments entirely dark brown ; segments 4 to 10 sub-cylindrical, measuring in the single specimen from 15 by 8 units to 19 by 7 units ; segments 11 to 15 elongate, sub-equal, about 29 to 30 by 6 to 7 units, 15 terminating in a stout bristle, but without a stylet. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 measuring 147, 119 and 139 units respectively. Scutellum yellow ; bearing a transverse row of nine bristles, and about eleven small hairs. Wings yellowish, especially at their anterior borders. Macrotrichia fairly numerous at tip, in cells R_3 and M_1 , and a few in cell M_2 . Costa extending three-quarters of wing-length. First radial cell oval ; second large, over two and a half times as long as first. Hind legs with dark brown bands occupying distal third of femur and of tibia. Knees dark. The four anterior legs have similar but less distinct and extensive adornment. Hairs on femora and tibiae shorter, fewer spine-like. Hind femora not much wider than others. T.R. about 2. First tarsal segments armed with spines ; those of hind legs straight, less than one-third length of tibiae, with spines at base, apex, and on shaft. Fourth tarsal segments sub-cylindrical ; those on four anterior legs about half length of fifth segment, those on hind legs about as long as this segment. Claws on four anterior legs equal, small ; those on hind legs single and barbed (or perhaps unequal and fused basally), the one part small, the other about as long as fifth tarsal segment. Abdomen darker brown, less distinctly banded ; cerci darkish brown. Spermathecae two (and a rudimentary third), highly chitinated, oval, sub-equal, about 100 to 110 μ by 80 μ ; the duct chitinated for some distance, about 25 μ , and somewhat wider than in the following species.

Dun Mt., 3,000 ft., 5 to 7.i.1922, 1 ♀.

Monohalea ferruginea, sp.n.

Allied to *Monohalea antipodalis* I. & M., but smaller, with more extensive adornment of the legs, and differing also as indicated below.

FEMALE. Length of wing, 1.9 mm.; greatest breadth of wing, 0.67 mm.

Eyes bare, widely separated above. Antennae darkish brown, proximal segments pale brown at bases, the last five entirely darkish brown; segments 4 to 10 sub-cylindrical, measuring in one specimen from 12 by 7 units to 13 by 6 units; segments 11 to 15 elongate, measuring in the same specimen 21, 21, 24, 24 and 28 by 6 units, 15 with a stout terminal bristle but no stylet. The combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 in this specimen 119, 90 and 106 units respectively. Scutellum yellow, bearing six bristles and about the same number of small hairs. Wings yellowish, especially at anterior border, bearing macrotrichia at tip, a good many in cells R_5 and M_1 , and a very few in M_2 . Costa extending three-quarters of wing-length. First radial cell lenticular; second large, about three times as long as first. All legs with broad, dark brown bands on femora and tibiae, those on femora not reaching quite to knees, those on tibiae at apices. On hind legs the dark bands occupy the greater part of femora, and the distal third of tibiae; and on four anterior legs are rather indistinct and diffuse. Hairs on femora and tibiae shorter, fewer spine-like. Hind femora not much broader than others. T.R. about 2. First tarsal segment of hind legs without spines on middle portion of shaft, not much curved, a little over one-third the length of tibia (in male *M. antipodalis* it is about one-quarter the length of the tibia). Fourth tarsal segment shorter, bell-shaped and about half length of fifth on the four anterior legs; cylindrical and about as long as fifth on hind legs. Claws on four anterior legs equal, small; those on hind legs single and barbed (or perhaps unequal and fused basally), the one part small, the other longer than fifth tarsal segment. Abdomen with distal segments distinctly banded; cerci pale brown. Spermathecae two (and a rudiment of a third), highly chitinated, roughly oval, large, sub-equal, about 120μ by 78μ ; the commencement of duct curved, chitinated for some distance, about 30 to 35μ .

Waiho, 18.i.1922, 1♀ (discoloured); and 30.i.1922, 1♀.

PALPOMYIA (Mg.), Kieff.

All the four species of *Palpomyia* represented in the collection are somewhat similar, very dark brown insects, with a scutal tubercle, only the fore femora armed, and the fifth tarsal segment without batonnets. They may be distinguished by superficial characters as shown in the key.

KEY TO THE SPECIES OF *Palpomyia*. FEMALES.

1. Tibiae of hind legs entirely dark brown ; gland rods on 5-7 tergites *nelsoni* sp.n.
 Tibiae of hind legs yellowish-brown ; gland rods on 3-7 tergites *rastellifer* sp.n.
 Tibiae of hind legs dark brown at the apex 2
2. Fore femora with dark band about middle ; gland rods well developed, on 5-7 tergites at least ; spermathecae with duct chitinised for short distance *cantuaris* I. & M.
 Fore femora without dark band in middle ; gland rods poorly developed, small on 6-7 tergites ; spermathecae with duct hardly at all chitinised *urpicifemoris* sp.n.

Palpomyia cantuaris I. & M.

Wairakei, 6.iii.1923, 1 ♀ ; Lake Rotoroa, 13 to 20.i.1928, 2 ♀♀.

Palpomyia nelsoni, sp.n.

A very dark brown, almost black species, with a small scutal tubercle, unadorned wings, dark brown halteres, femora and tibiae of four posterior legs almost entirely dark brown, fore femora swollen, only the fore femora armed with spines, and the fifth tarsal segments without batonnets ; resembling in many respects the Chilean species *P. subfuscula* I. & M., but differing as indicated below.

MALE and FEMALE. Length of wing : female, 2.3 mm. ; male, smaller, 1.5 mm. Greatest breadth of wing : female, 0.76 mm. ; male, 0.48 mm. The single male was not only smaller than the females, but also distinctly paler.

Head similar. Antennae in the single male with lengths of last five segments 13, 15, 27, 37 and 40 units respectively ; and in female with combined lengths of segments 11 to 15, 4 to 10, and 3 to 10 in one specimen 220, 101 and 126 units respectively.

Thorax similar. Scutellum bearing six stout bristles in female, four in male.

Wings similar, but tip rounded ; second radial cell longer, in

male about 2.5 times, in female over 3.5 times as long as first; and fork of median vein nearer cross-vein, the distance from fork to cross-vein less than length of cross-vein. Halteres with dark brown knobs.

Legs similar, but fore legs usually with femora paler at base and tibiae not so dark as those of the four posterior legs. Fore femora armed with rather more numerous spines, namely, eight or nine in male, about thirteen in female. T.R. about 2.

Abdomen similar, but paler markings on tergites less distinct. Spine-like processes present on anterior margins of the fifth, sixth and seventh tergites. Spermathecae two (and a rudimentary third), highly chitinised, oval, rather unequal, in one specimen measuring about 103μ by 75μ and 77μ by 63μ respectively, the commencement of duct chitinised for about 10μ . The hypopygium is missing from the single male.

Nelson, 3.xii.1921, 1 ♂ (damaged) and 14.xi.1923, 1 ♀ (with pupal pelt); Waiho, 16 and 28.i.1922, 2 ♀♀; Aniseed Valley, 1 to 4.xii.1923, 1 ♀. The association of the male with the females is purely conjectural, and may be erroneous.

Palpomyia rastellifer, sp.n.

A very dark brown species of medium size, with a small scutal tubercle, unadorned wings, dark brown halteres, yellowish legs, only the fore femora armed with spines, and the fourth and fifth tarsal segments unarmed.

FEMALE. Length of wing, 2 mm.; greatest breadth of wing, 0.68 mm.

Head very dark brown. Eyes bare, separated above by a wedge-shaped area. Mandibles armed with seven strong teeth. Palpi pale brown, the last segment somewhat infuscated; third segment almost cylindrical, without a sensory pit; lengths of last three segments in one specimen 14, 12 and 14 units respectively. Antennae with torus yellowish, segments 3 to 10 mainly pale brown but darkened at apex, and segments 11 to 15 almost entirely dark brown; segments 4 to 10 sub-cylindrical, sub-equal, about 12 or 13 by 5 or 6 units; segments 11 to 15 much elongated, sub-equal, about 40 to 47 by 5 units, 15 without a stylet. The combined lengths of segments 11 to 15, 4 to 10 and 3 to 10 in one specimen measuring 217, 93 and 113 units respectively.

Thorax very dark brown, shining. Scutum armed anteriorly with a small pointed spine, and bearing three or four spine-like bristles just above wings. Scutellum and postscutellum very dark brown, the former bearing four stout, dark bristles.

Wings much as usual, unadorned, but with veins and anterior margin a little infuscated; without macrotrichia. Tip rounded. Costa extending about five-sixths of wing-length. Radial cells large: first almost rhomboidal, about five times as long as broad; second about three times as long as first. In one of the specimens R_2 is absent from one wing—a not uncommon abnormality. Median vein sessile, the distance from fork to cross-vein about equal to length of cross-vein. Fork of Cu slightly distal to level of cross-vein. Halteres with dark brown knobs.

Legs almost uniformly yellowish-brown, but the coxae dark brown, knees, and tibio-tarsal joint on hind legs, a little darkened, and last 2 to 3 tarsal segments on all legs infuscated. Femora of fore legs a little swollen, armed with about ten spines. Other femora, and all tibiae, slender, unarmed. T.R. about 2.3. Third tarsal segment much shorter than second; fourth almost cordiform, unarmed; fifth longer, slender, unarmed. Claws normal, small, equal, barbed.

Abdomen very dark brown. Spine-like supports for eversible glands present on the third to seventh tergites. Spermathecae two, highly chitinised, sub-equal, oval, about 75μ by 60μ , the commencement of duct chitinised for about 7μ or less.

Lake Brunner, 3.ii.1922, 1 ♀; Waiho, 19.i.1922, 1 ♀.

This species resembles a little, perhaps, the European *P. luteifemorata* Edw., in which, however, the hind tibiae and tarsi are black.

Palpomyia urpicifemoris, sp.n.

A dark brown species of medium size, with a scutal tubercle, unadorned wings, dark brown halteres, brown legs with dark markings at the apices of the femora and tibiae, the fore femora much swollen and armed with spines, and the fourth and fifth tarsal segments unarmed.

FEMALE. Length of wing, 2.7 mm.; greatest breadth of wing, 0.85 mm.

Head dark brown. Eyes bare, separated above by a wedge-

shaped area. Mandibles with about a dozen teeth. Palpi darkish brown; lengths of last three segments in the unique specimen about 22, 13 and 17 units respectively, the third almost cylindrical, without a pit. Antennae with torus darkish brown, segments 3 to 10 with pale brown basal and dark brown apical halves, segments 11 to 15 entirely dark brown: segments 4 to 10 sub-cylindrical, ranging from 17 by 7 units to 19 by 6 units; segments 11 to 15 elongate, sub-equal, about 37 to 44 units by 5 units, 15 without a stylet. The combined lengths of segments 11 to 15, 4 to 10 and 3 to 10 measuring 198, 117 and 144 units respectively.

Thorax dark brown, with a grey pruinescence. Scutal tubercle small, pale, pointed. Scutellum dark brown, but not so dark as scutum; bearing four stout, dark bristles. Postscutellum dark brown.

Wings much as usual, unadorned, without macrotrichia. Costa extending four-fifths of wing-length. First radial cell almost triangular, second about three and a half times as long as first. Median vein sessile, the distance from fork to cross-vein barely as long as cross-vein. Fork of Cu at about same level as cross-vein. Halteres with brown knobs.

Legs brown, with coxae, knees, apical quarter of all tibiae and of four posterior femora dark brown, and last 2 to 3 tarsal segments infuscated. Fore femora not distinctly adorned, but their distal halves more or less infuscated. Fore femora much swollen, armed with fifteen to eighteen spines; fore tibiae curved, with the usual apical spine; other femora and tibiae slender, unarmed. T.R. about 2.4. Tarsal segments normal, unarmed. Claws normal, small, equal, barbed.

Abdomen dark brown, tergites of basal segments paler brown in middle posteriorly. Spine-like supports for eversible glands poorly developed, present but small on sixth and seventh tergites. Spermathecae two, highly chitinised, sparsely pitted, oval, rather unequal, but partly collapsed in the specimen, about 70 to 80 μ by 60 to 70 μ ; the duct hardly at all chitinised.

Kaikoura, 24.ii.1922, 1 ♀.

This species apparently resembles somewhat the European *P. ephippium* (Zett.) (*P. rubra* Kieff.) in which, however, there are no gland-rods, and the halteres are yellowish or white.

UNUSUAL DEVELOPMENT OF RABIES SYMPTOMS IN MAN

BY

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This article is prompted by the recent occurrence in Palestine of two cases of hydrophobia, which, from the appearance of symptoms in one case twelve months after the date of infection, and in the other four months after mere contact with the saliva of a herbivorous animal, appear to us to be of sufficient interest and rarity to merit description and discussion.

I. PROLONGED INCUBATION PERIOD OF RABIES IN MAN.

(1) *History of present case.*

A.B., male, aged 30 years, Arab landowner, was on 30.ix.30 bitten with moderate severity on the dorsum of the right hand by a dog which, from later laboratory examination, was proved to have been rabid at the time of biting. There was, unfortunately, no local cauterisation performed, but between the 1st and 14th days of October a complete course of anti-rabies injections was administered. The vaccine employed was a 2 per cent. emulsion of fixed virus (rabbit brain) killed by the combined action over 24 hours of heat at 37° C. and of 1 per cent. phenol, and diluted to half with physiological salt solution prior to use; treatment consisted of the daily intracutaneous administration, over fourteen consecutive days, of 4 c.cm. of this killed carbolised virus. Three months after the last injection the patient reported to the district medical officer for final observation, and was then certified as being in good health. On the evening of 1.x.31, however, exactly one year after the date of bite, while on a visit to a neighbouring farm, he experienced a severe shock on seeing, as he supposed, a snake coming in his

direction along the path on which he was riding. During the next three days he appeared to be preoccupied and out of sorts, and, on his return home four days afterwards, complained of being tired and of a stiffness in his neck and right shoulder. The following morning he became a prey to unaccustomed and unaccountable fears and in the afternoon developed along with an increasing agitation a definite 'hydrophobia'; somewhat later, marked dysphagia, aerophobia, and all the classical signs of medullary involvement contributed to a clinical picture of true rabies, and death supervened on 8.x.31, six hours after admission to hospital in Jerusalem.

(2) *Discussion*

A study of available statistics relating to the incubation period of hydrophobia in man affords proof of its extraordinary variability. According to Harvey and McKendrick (1930), the period of incubation varies with the distance to be travelled by the virus. For implantation on the head it is approximately 27 days, on the arm 32 days, and on the leg 64 days; but these figures are subject to wide variation. Koch (1931), appraising Bauer's statistics based on 537 untreated cases, shows the mean incubation time to be 126 days; but, if from these calculations there be excluded 17 cases with a latent period of more than one and a half years, and 10 cases which are frankly dubious, the average incubation period for the remaining 510 cases is to be reckoned as only 72 days. Bauer's figures also go to show that nearly 90 per cent. of these deaths occurred within five months of the date of infection. Again, much valuable information may be derived from an analytical review by McKendrick (1930) of the statistical data furnished by 31 Pasteur Institutes, which submitted to the Health Organisation of the League of Nations results of anti-rabies treatment for 1927 or 1928 on the uniform plan recommended at the International Rabies Conference of 1927. From this source it will be learned that, of 150 cases of hydrophobia which occurred among 31,656 persons treated and concerning which accurate records had been kept, the average interval between infection and death was 52 days, with extreme limits of 12 and 275 days. Further, it emerges that, among 111,427 patients treated at the Pasteur Institutes of the Union of Soviet Socialist Republics

during 1927, rabies supervened in 163 cases, after incubation periods varying between 10 and 444 days but averaging 56. In this connection it is of interest to compare incubation times among treated and untreated populations; thus, of the 190, 98 and 60 untreated cases of hydrophobia collected respectively by the Comité d'Hygiène (France), Hoegyes (Hungary) and Nitsch (various sources), 88, 81·64 and 60 per cent. occurred within three months of the date of bite, while among the same cases the percentage figures within the first six months proved to be 99, 94 and 92. Again, of the 207, 106 and 133 rabies deaths reported respectively by the Pasteur Institutes of Paris, Hungary and Kasauli, 86·47, 90 and 94 per cent. took place during the first three months, and 94·2, 94·36 and 97·75 within the first six months after exposure to infection. A scrutiny of these figures amply corroborates the statement of Hoegyes (1900) that 'an incubation period of more than three months is a rarity, while one of more than six months is altogether exceptional.' The deductions of Harvey and Acton (1923) made from similar data likewise receive support: generally speaking, 'the treated population has a somewhat shorter incubation period than the untreated one, due in all probability to the inclusion of greater numbers of the badly wounded in the treated population.'

While, however, in the vast majority of cases symptoms develop in from 30 to 180 days after infection, there have been recorded from time to time noteworthy instances of remarkably short and long incubations. As examples of short incubation periods, Bauer's statistics show rabies to have supervened in 8·24 per cent. of his cases during the first 19 days following the bite; Konradi (1923) has recorded periods of 12, 13 and 14 days; Alivisatos (1926) periods of 10 and 13 days; McKendrick (1930), in the analytical review already mentioned, periods of 10, 11, 12 and 13 days; while Hoegyes (1900) states that Jouffroy, Tardieu, Bouley and himself have each encountered cases of 13 days. Moreover, Babes (1912) believes the percentage of cases wherein hydrophobia supervenes before the fifteenth day after the bite to be not more than 5 per cent. of the total. It is the opinion of Lubinski and Prausnitz (1926), of Alivisatos (1926), and of others, including ourselves (1931), that such cases may well be explained by an increased virulence of rabies virus. As instances of prolonged incubation periods,

Hoegyes (1900) and Babes (1912) cite cases ranging from 230 days up to several years, McKendrick (1930) cases of 390 and 444 days. While Osler (1921) considers the question of prolongation for a year or even two years not to have been definitely settled, Rosenau (1927) contends that 'the prolonged and variable period of incubation is due in part to the fact that it takes time for the virus to travel along the nerves to the central nervous system, and that it may remain there dormant (latent) until conditions favour multiplication.' Further, Remlinger (1910) bases his explanation of such cases on the assumption that the virus can vegetate, without causing injury at the seat of entry or in the central nervous system, until it is roused to activity by one of the following causes: nervous shock, terror, psychic influences, trauma, over-exertion, chill or abuse of alcohol. (As regards cases with incubations of more than a year or two, Lubinski and Prausnitz (1926) express the view that new infections may possibly have taken place in the interval.) According to Babes (1912), there would appear to exist during the months following Pasteurian treatment a state of unstable equilibrium between the rabies organism and the anti-virus, but the balance may readily be upset as a result of mental or physical stress, when the virus gains the upper hand and proceeds towards normal reproduction. Moreover, although rabies patients often assert that the onset of symptoms was consequent upon shock, fear, trauma or chill, and thus apparently confirm Remlinger's hypothesis, it has to be considered whether those very emotional and physical disturbances incriminated in the production of symptoms may not themselves be actually the first manifestations of the disease.

The theory of latency of virus, however, receives considerable practical support from the recorded experiences of De Mello (1924), Herrmann (1925), Panisset and Verge (1925), Thiéry (1929) and others. Thiéry, for example, gives an account of a dog which, in the absence of any admissible evidence to the contrary, must have been a carrier of rabies virus in the latent state; it was shown that a woman, who afterwards died of hydrophobia and contained typical Negri-bodies in her brain, had been bitten by this animal, which had no symptoms of rabies at the time of biting and developed none later. Latency of virus can also afford the sole explanation of the rarely described *Lyssa recurrens*—a condition wherein rabies

in the biting animal seems to run a recurrent course. While, then, it is true that the occurrence of such cases must, on account of their extreme rarity and of the necessary lack of absolute proof, be viewed with varying degrees of scepticism by conservative workers, it is, on the other hand, difficult, well-nigh impossible, to account for protracted periods of incubation, unless latency of virus be allowed. Thus Rochaix (1924), in the case of a patient who, bitten on the hand by a cat, showed, one year after completion of treatment and after a preliminary pleuro-pneumonia, unmistakable symptoms of hydrophobia and whose medulla later afforded proof by animal experiments of the presence of rabies virus in non-attenuated form, finds an explanation of the delay in the appearance of symptoms in Remlinger's statement that the virus may remain latent in the nerve centres for long periods of time, and may thereafter be brought into activity by secondary influences such as trauma, cephalitis, emotion, cold, etc., the cause of onset in this instance being ascribed to chill. In respect of the case described in this article, it seems to us also not to be unreasonable to seek an explanation of the delay along identical lines, the predisposing factor here, however, being the shock to the patient's nervous system given by the unexpected appearance of a snake.

II. RABIES TRANSMITTED TO MAN BY HERBIVORA

(1) *History of present case.*

A.H., male, aged 48 years, of Russian extraction and practising as a pharmaceutical chemist in a small township, was on 30.iv.31 called in to attend a sick calf, which had developed symptoms suggestive of rabies, viz., difficulty of swallowing and progressive weakness in the extremities. Some days later the clinical diagnosis was confirmed by laboratory examinations, which afforded in rabbit series a positive biological test, and demonstrated in the hippocampus Negri-bodies in considerable number. Despite the diagnosis, which was immediately communicated to all concerned, and in spite of his repeated manipulations to alleviate the dysphagia, the chemist sought neither advice nor treatment, his hands apparently not having received obvious injury. On 31.viii.31, however, the onset of rabies was heralded by loss of appetite, slight fever, pains

in the arms and moderate paresis of the extremities. Next day, after a short period of marked depression, the patient exhibited signs of increasing disquietude. He had attacks of vomiting, dysphagia, aerophobia and true hydrophobia, all of which contributed to a state of continued mental and physical unrest. Later, after having become definitely aggressive towards his attendants and bent on self-destruction, he died in deep coma on 3.ix.31. With regard to this patient, it seems right to emphasize that at no time previously had he been exposed to any risk of infection with rabies virus.

(2) *Discussion.*

According to Harvey and McKendrick (1930), rabies is confined to a comparatively small number of animal species, so far as it has come under observation. It is essentially a disease of canine animals, and is conveyed by them to a number of other animals. Now in the infection of man, the dog undoubtedly plays the chief rôle, but transmission is not infrequently effected by other carnivores such as jackal, wolf and fox among the Cynoidea, cat, civet and hyaena among the Aeluroidea—the two sections of Carnivora Vera most intimately concerned with the spread of the disease. Rabies infection in man through the bite of rabid herbivores is, on the other hand, exceedingly rare, and such instances as have occurred have usually been made the subject of special record by, for example, Remlinger (1905) and Koch (1913). In an evaluation of the comparative danger of bites inflicted by animals of whatever classification, Rosenau (1927) expresses the general opinion in his statement that wolf-bites are most to be feared, on account of the savage character of the wound and the virulence of the virus; in order of relative danger the bites of other animals are to be placed as follows: cats, dogs, foxes, jackals, horses, asses, cattle, sheep, pigs. Further, it is held that the bites of horses and other herbivora are less dangerous because their blunt teeth usually cause contused wounds without breaking the skin. In this connection, however, a true conception of the absolute and relative importance of the biting animals known to be most concerned in the dissemination of rabies, can only be formed from a perusal of relevant statistics.

Thus in France during the period 1850-1876, when no specific

anti-rabies treatment was available, there occurred 770 cases of human rabies ; of this number, dogs were responsible for 707 deaths, or 91·8 per cent., cats for 23 or 3 per cent., wolves for 38 or 4·94 per cent., while foxes and cattle each accounted for 1 death of 0·13 per cent. Again, a Table prepared by Hoegyes (1900), to show the number of bitten persons attending for Pasteurian treatment at the Institutes in Paris (1887-1895) and in Budapest (1890-1895), classifies the attending populations according to the species of the biting animal, and clearly indicates the relative responsibility of each species. Unfortunately, in this Table, which is given below in modified form, mortality figures are not included.

TABLE I (after Hoegyes)

Species of biting animal	Bitten persons treated at Pasteur Institutes and classified according to animal species incriminated			
	At Paris		At Budapest	
	Total treated	Percentage	Total treated	Percentage
Dogs	13,315	93·13	4,481	90·32
Cats	823	5·75	387	7·80
Wolves	17	0·12	19	0·38
Foxes, Jackals	7	0·04	1	0·02
Solipeds	59	0·40	17	0·34
Ruminants	68	0·46	43	0·86
Other species	7	0·04	13	0·26
Total	14,296	...	4,961	...

Of interest also in this respect, are statistics of 22,519 persons treated at Kasauli ; of the total number attacked by presumably rabid animals, the following percentages indicate responsibility : dogs 80·8, jackals 18·5, horses 0·5, foxes, cattle, hyaenas, camels, goats and deer less than 0·4 each.

Some interesting facts regarding the bites of herbivora, and germane to the present discussion, were recorded from Russia by Ouchakoff (1923). During the period 1902-1914 the registers from all the Russian anti-rabies stations showed 3,522 treated patients to have been bitten by rabid cattle, with no ensuing mortality, and

2,859 by rabid horses with one death. In his comments on these figures, McKendrick (1924) states: 'It is usually held that rabies cannot be conveyed by the bites of rabid herbivora, but statistics of any magnitude have been difficult to obtain. The value of the Russian figures is that they set an upper limit to the chance of infection. If it exists at all it is less than one-sixtieth per cent.' Recently valuable contributions have been made to our knowledge of the subject of animal responsibility by the statistical data emanating from 31 anti-rabies institutes, wherein 31,656 bitten persons received treatment during 1927 and 1928, and from the Pasteur Institutes of the Union of Soviet Socialist Republics, which dealt with 72,632 patients during 1927. As previously mentioned, these statistics have formed the subject of McKendrick's 'Analytical Review' (1930), and from the figures supplied we have prepared Tables II and III, which show the treated patients analysed and classified according to the species of biting animal.

TABLE II

Embodying the statistics of thirty-one institutes, but excluding the Russian figures.

Species of biting animal responsible	Statistics of persons treated.			
	No. of persons bitten	Percentage of total number	No. of deaths	Percentage mortality
Dogs	26,527	83·8	112	0·42
Cats	1,532	4·8	0	0
Wolves	71	0·2	13	18·31
Jackals, Hyaenas	1,965	6·2	31	1·58
Solipeds	105	0·3	0	0
Ruminants	819	2·6	0	0
Other animals	637	2·0	0	0
Totals	31,656	...	156	...

From the foregoing, it will be seen that even after actual bites have been inflicted by herbivores, hydrophobia rarely if ever supervenes; its occurrence, therefore, as in the present case, after mere contact with the saliva of a rabid calf must surely be altogether unusual. That it can, however, occur at all is obviously of consider-

able importance in expressing an opinion as to the necessity of submitting to anti-rabies treatment persons whose skin has come into contact with the saliva of a rabid animal, if there be no visible break in the continuity of the skin surface. Now in this connexion Remlinger (1905) found, during his experimental work, that rabies could be induced in rabbits and guinea-pigs by bandaging the virus upon the freshly shaven skin, and Galli-Valerio (1906) confirmed these results soon afterwards. Again Rosenau (1927) discovered that rubbing the virus into the scarified skin was, next to subdural inoculation, one of the most reliable methods of transmitting the

TABLE III

Embodying the statistics of the Russian Pasteur Institutes for 1927

Species of biting animal responsible	Statistics of persons treated			
	No. of persons bitten	Percentage of total number	No. of deaths	Percentage mortality
Dogs	64,384	88.7	135	0.15
Cats	5,562	7.7	2	0.03
Wolves	406	0.6	35	7.35
Cattle and Horses	1,341	1.8	0	0
Other animals	939	1.2	0	0
Totals	72,632	...	172	...

disease. These experiments are held by Rosenau to explain the possibility of infection from insignificant bites, as well as infection following exposure without biting, as in licking. 'It can be asserted,' says Marie (1927), 'that the saliva of a rabid animal contains the virus in a form incomparably more active than does the nervous system. Thus, as Remlinger has observed, it is only with difficulty that one can give rabies by cutaneous wounds with an emulsion of virulent brain, whereas clinical experience frequently shows cases of hydrophobia among persons, in whom an extremely small abrasion of the skin or mucous membrane has been contaminated with saliva.'

These opinions, based as they are on experimental and clinical evidence alike, seem to permit of but one answer to the question asked at the International Rabies Conference of 1927: 'Do you recommend vaccination in the case of a person who has run the risk of infection only by having the skin contaminated with the saliva of a rabid animal?'

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P. MALARIAE IN FREETOWN, SIERRA LEONE

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I. INTRODUCTION

The three recognised human species of the genus *Plasmodium* are present throughout the West African Colonies, but the available literature concerning malaria in these colonies shows that their proportions vary considerably. Below in Table I are summarised the more important papers, in which sufficiently large figures are quoted.

It will be noted from this table that figures, even from the same districts and compiled by the same observers, show considerable variation. Thus the figures given by Blacklock and Gordon (1925) show the infection rate of the native population in Freetown as 21 per cent. in one set of figures, and 5 per cent. in another, the difference being due to the fact that the higher percentage was derived from the examination of children and the lower from adults. Unfortunately, certain information is lacking from many of the records quoted; for example, frequently the technique (thick or thin film) employed in the examination is not stated, and in some instances the ages of the groups examined are not given, in others the nationality. In considering the table, therefore, due allowance must be made for the variations consequent on these differences.

The papers quoted in the table show that the Colonies of Senegal, French Niger and French Guinea have a far higher proportion of quartan infection (23 per cent. to 55 per cent. of the total infections) than is found in any of the other West African Colonies. In the British West African Colonies considerable variation occurs. In

TABLE I

Showing the distribution of malaria species in the West African Colonies.

Country	Authority	Total examined	Total positive	Percentage positive	<i>P. falciparum</i>		<i>P. malariae</i>		<i>P. vivax</i>		Mixed infections		Un-diagnosed	
					Total positive	Percentage positive	Total positive	Percentage positive	Total positive	Percentage positive	Total positive	Percentage positive	Total positive	Percentage positive
Portuguese Guinea	Barreto (1927) ...	336	156	46	72	46	4	3	75	48	5	3	0	0
Senegal, French Niger and French Guinea	Bouffard (1909) ...	347	265	73	24	9	127	48	17	6	11	4	86	32
	Leger (1914) ...	1721	1284	75	461	36	709	55	114	9	0	0	0	0
	Leger and Baurly (1922a) ...	366	155	42	104	67	42	27	9	6	0	0	0	0
	Leger and Baurly (1922b)	940	328	35	195	59	126	39	7	2	0	0	0	0
	Leger, Pinaud and Bédier (1923)	250	167	67	111	66	49	29	1	1	6	4	0	0
	Leger, Bédier and Baurly (1923)	690	441	64	303	69	133	30	5	1	0	0	0	0
	Leger and Nogue (1923) ...	890	328	37	252	77	74	23	2	0	0	0	0	0
	Leger and Bédier (1924)	210	102	49	68	67	33	32	1	1	0	0	0	0
	Bédier, Laurency and Baurly (1924)	135	90	67	58	64	27	30	3	3	2	2	0	0
Sierra Leone ...	Durieux and Sall (1929) ...	153	37	24	27	73	10	27	0	0	0	0	0	0
	Butler (1915) ...	100	49	49	41	84	8	16	0	0	0	0	0	0
	Dalziel and Johnston (1915)	342	201	59	191	95	9	5	1	1	0	0	0	0
	Blacklock and Gordon (1925)	461	221	48	171	78	46	22	1	0	3	1	0	0
	Macdonald (1926) ...	809	169	21	149	88	12	7	2	1	5	3	1	1
	'Ann. Med. San. Rep.' (1928)	366	18	5	14	78	0	0	1	6	0	0	3	17
	'Ann. Med. San. Rep.' (1929)	1059	542	51	439	81	84	16	11	2	3	1	5	1
	'Ann. Med. San. Rep.' (1929)	529	158	30	153	97	0	0	5	3	0	0	0	0
	'Ann. Med. San. Rep.' (1929)	2123	524	25	509	97	3	1	12	2	0	0	0	0
Gold Coast ...	Coghill and Hänschell (1915)	462	211	46	159	75	26	12	15	7	11	5	0	0
	'Ann. Med. San. Rep.' (1928)	606	259	41	142	55	35	14	22	9	60	23	0	0
	Butler (1929) ...	217	35	16	35	100	0	0	0	0	0	0	0	0
	Butler (1929) ...	1041	504	48	473	94	22	4	9	2	0	0	0	0
Nigeria ...	Butler (1929)	328	59	18	59	100	0	0	0	0	0	0	0	0
	Butler (1927) ...	2285	1015	30	947	93	68	7	0	0	0	0	0	0
	Morrison (1928) ...	1519	290	19	274	94	15	5	1	0	0	0	0	0
	'Ann. Med. San. Rep.' (1928)	3623	809	22	749	93	60	7	0	0	0	0	0	0
	'Ann. Med. San. Rep.' (1929)	4676	797	17	779	98	18	2	0	0	0	0	0	0
	'Ann. Med. San. Rep.' (1929)	1563	185	12	167	90	16	9	2	1	0	0	0	0
	'Ann. Med. San. Rep.' (1929)	4030	1062	26	1035	97	26	3	0	0	0	0	0	0
	'Ann. Med. San. Rep.' (1930)	2444	396	16	396	100	0	0	0	0	0	0	0	0
	'Ann. Med. San. Rep.' (1930)	1318	194	15	173	89	20	10	1	1	0	0	0	0
	'Ann. Med. San. Rep.' (1930)	5548	1667	30	1627	98	40	2	0	0	0	0	0	0
Cameroons, French Equatorial Africa, French Sudan	Clapier (1919) ...	500	285	57	121	42	24	8	140	49	0	0	0	0
	Gambier (1922) ...	255	201	79	197	98	3	2	1	0	0	0	0	0
Congo ...	Lebouef (1918) ...	71	21	30	8	38	2	10	11	52	0	0	0	0
	Ledentu and Vauzel (1927)	475	475	100	161	34	22	5	242	51	0	0	50	10
	Schwetz and Baumann (1929)	952	814	85	557	69	195	24	0	0	62	8	0	0

Nigeria exact records for the years 1928, 1929 and 1930 are available and show a quartan incidence varying from 0 per cent. to 20 per cent. of the total infections; the only report in which quartan was above 10 per cent. came from Calabar. In the Gold Coast similar figures for the years 1928 and 1929 were respectively 0 per cent. and 4.4 per cent., though in this colony two sets of observations in 1915 gave figures of 12.3 per cent. and 13.5 per cent. In Sierra Leone six sets of figures are available, all for Freetown, covering the years 1915, 1925, 1926, 1928 and 1929. In these the quartan percentages vary from 0 per cent. to 21.9 per cent. of the total infections.

The 1931 observations recorded here were conducted during the months of February to September, and the examination of the blood of native children was carried out by means of thick films. In some instances the diagnosis by thick films was doubtful, and in as many of these cases as possible the child was re-examined by means of thin films.

II. THE INCIDENCE, DISTRIBUTION AND TYPE OF *P. MALARIAE* IN FREETOWN, IN 1924 TO 1926

For the years 1924-26, two sets of figures from this laboratory are available. Blacklock and Gordon (1925) examined a series of 809 children aged up to two years, of whom 20.9 per cent. were infected; 88.2 per cent. of these infections were due to *P. falciparum*, 7.1 per cent. to *P. malariae*, 1.2 per cent. to *P. vivax*, 2.9 per cent. to mixed infections, the remaining 0.6 per cent. being undiagnosed. Macdonald (1926) examined 1,059 children, aged 3 to 13 years, of whom 52.3 per cent. showed parasites in the peripheral blood. Of these positive cases 79.7 per cent. were due to *P. falciparum*, 15.7 per cent. to *P. malariae*, and 0.8 per cent. to *P. vivax* and undiagnosed infections. Unfortunately we are unable to quote the localities in which occurred the eighty-six quartan cases mentioned by Macdonald, but exact addresses of the fifteen cases mentioned by Blacklock and Gordon are available. After the publication of this latter paper the work was continued for several months and a further eleven cases were obtained from Freetown. The site of occurrence of these twenty-six cases is shown on the attached map. It will be noted that they are found mainly in the eastern and western portions

of the town, the centre of the town being free from the disease, so far as can be judged from such a small figure as some 260 persons examined. It can also be seen that the cases tend to occur along the anopheline infected streams described by Blacklock and Evans (1925), the main focus lying close to Alligator Brook on the west of the town.

The type of parasite found in these quartan infected cases was of the text-book *P. malariae* type, i.e., did not resemble the *P. ovale* of Stephens (1922).

III. THE INCIDENCE AND TYPE OF *P. MALARIAE* IN 1931

During the latter part of 1930 we noticed an apparent increase in the proportion of cases of quartan malaria. In February, 1931, we commenced a systematic examination of the native population, which, for the sake of convenient comparison with the earlier findings in 1924 to 1926, we confined to children attending the infant clinic or the urban schools. It is advisable, however, to mention here that the increase in quartan infections, which we will show to have occurred among these children, is almost certainly shared by the adult native and European populations. Manson-Bahr (1929) refers to the fact that in Macedonia during the war quartan malaria remained confined to the children in the villages, and did not spread to the English troops quartered in the district, although *P. falciparum* and *P. vivax* were readily transmitted to them. Figures regarding the adult natives of Freetown are not yet available, but amongst thirty-five Europeans in whom malaria parasites were found, seven were found to be infected with *P. malariae*. This figure although very small is significant, for previous to 1931 quartan malaria was almost unknown amongst Europeans living in Freetown.

Below, in Table II, we contrast the incidence of *P. falciparum*, *P. malariae* and *P. vivax* amongst children in 1924-26 and 1931.

It can be seen in Table II that not only the figures given for 1924-25 by Blacklock and Gordon, but our own figures show a considerably lower proportion of children infected with malaria parasites than do Macdonald's figures. This is probably due to the season of the year in which the cases were examined, for Blacklock and Gordon (1925), whose observations covered an entire year, have shown that by far the greatest incidence of malaria occurs in Freetown

during the months of July to February inclusive. Macdonald's observations were all conducted during this very period, whereas our observations extended from February to early in September and therefore did not include four of the months of greatest malaria incidence. The increase shown by our figures over those of Blacklock and Gordon is almost certainly due to our greater use of the thick film.

TABLE II

Showing the incidence of *P. falciparum*, *P. malariae* and *P. vivax* amongst 3,037 native children examined in Freetown during 1924-26 and 1931.

Authority ...	Under 3 years of age				3 to 14 years of age			
	1924-25		1931		1925-26		1931	
	Blacklock and Gordon		Gordon and Davey		Macdonald		Gordon and Davey	
Total examined ...	809		348		1059		821	
	Total	Per-centage	Total	Per-centage	Total	Per-centage	Total	Per-centage
Positive ...	169	20.9	143	41.1	542	51.1	323	39.3
<i>P. falciparum</i> ...	149	18.4	83	23.8	439	41.4	132	16.1
<i>P. malariae</i> ...	12	1.5	38	10.9	84	7.9	161	19.6
<i>P. vivax</i> ...	2	0.3	6	1.7	11	1.0	1	0.1
Mixed infections ...	5	0.6	*16	4.6	3	0.3	*23	2.8
Undiagnosed infections ...	1	0.1	0	0.0	5	0.5	6	0.7

* Of the 39 mixed infections quoted for 1931, 30 were mixed *P. falciparum* and *P. malariae*, the remaining one being infection with *P. falciparum* and *P. vivax*.

The most striking fact that emerges from these figures is the marked increase in *P. malariae* in both age groups. This increase, which appears to occur almost entirely at the expense of the *P. falciparum* infections, becomes more evident if the mixed infections are re-distributed according to their species (i.e., a triple infection in one individual appearing as one positive under each of the three headings, *P. falciparum*, *P. malariae* and *P. vivax*), and the figures expressed as percentages of the total positives. This has been done in Table III.

Table III shows that in 1924-26 the proportions of *P. falciparum* in the two age groups were respectively 91 per cent. and 81 per cent., whereas in 1931 they had dropped to 69 per cent. and 48 per cent. This loss has been replaced by a corresponding increase in the *P. malariae* percentage, which has risen from 9 per cent. and 16 per

TABLE III

Showing the proportions of *P. falciparum*, *P. vivax* and *P. malariae* amongst 1,177 native children found positive in Freetown during 1924-26 and 1931.

	Under 3 years of age		3 to 14 years of age	
	1924-25	1931	1925-26	1931
	Blacklock and Gordon	Gordon and Davey	Macdonald	Gordon and Davey
Authority				
Total positive	169	143	542	323
Percentage positive :				
<i>P. falciparum</i>	91.1	69.2	81.5	47.9
<i>P. malariae</i>	8.9	37.1	16.1	56.9
<i>P. vivax</i>	2.9	4.9	2.0	0.3

cent. to 37 per cent. and 57 per cent. The *P. vivax* infections remain almost negligible. This increase of quartan malaria is so marked as almost to justify the term 'quartan epidemic.'

None of the quartan infections examined were of the *P. ovale* type.

IV. THE DISTRIBUTION OF *P. MALARIAE* IN 1931.

During 1931 we examined 1,219 native children whose exact addresses were known and whose ages (with the exception of some half-dozen pupils of 16 to 19 years of age who happened to be attending the junior schools) varied from a few months to 15 years. Of these, 484 were positive, and in 478 a definite diagnosis was reached as to species, there being 259 cases of *P. falciparum* infection and 238 of *P. malariae*.

We have already referred to the fact that the twenty-six cases of quartan malaria noted by Blacklock and Gordon in 1924-25 were

mainly in the western and to a less extent in the eastern portions of the town, the centre of the town being apparently almost free from this infection, and we have indicated the exact position of these cases on the accompanying map. It was obviously impossible to adopt the same method in dealing with the large number of quartan cases noted in 1931. Recently the Colonial Government have published a road map of Freetown in which the city is divided into twenty-eight sections and these sections have been superimposed on the accompanying map. In Table IV, which must be read in conjunction with the map, we have compared the proportions of *P. falciparum* and *P. malariae* occurring in each of the nineteen districts from which our cases were obtained in the years 1924-25 and 1931.

Three facts can be deduced from Table IV. First, that quartan malaria, which in 1924-25 was confined to a few districts in the western and to a lesser extent in the eastern areas of the town, now in 1931 occurs in every district in which an adequate number of cases was examined. Secondly, that this universal increase is more marked in the western than in the central and eastern areas. It will be remembered that this western area harboured the majority of the few cases of quartan malaria recorded amongst the 1924-25 figures. Thirdly, that, in spite of certain irregularities in the figures, the tendency for quartan malaria to replace malignant tertian in contradistinction to appearing as a super-added infection, is constant in all areas and not confined to any particular district.

V. THE AGE INCIDENCE OF *P. MALARIAE* IN 1931

Blacklock and Gordon (1925), as a result of examining 800 native children up to the age of $2\frac{1}{2}$ years, showed that from the age of 1 month to 18 months there was a steady rise in the incidence of malaria, and from this period to the age of $2\frac{1}{2}$ years, at which their observations ceased, the malarial incidence remained almost constant. In 1926 Macdonald examined 722 children between the ages of 3 and 13 years in the endemic area of Freetown, and 337 children of the same ages in the hyperendemic area. The malaria incidence in these

TABLE IV

(To be read in conjunction with the Map.)

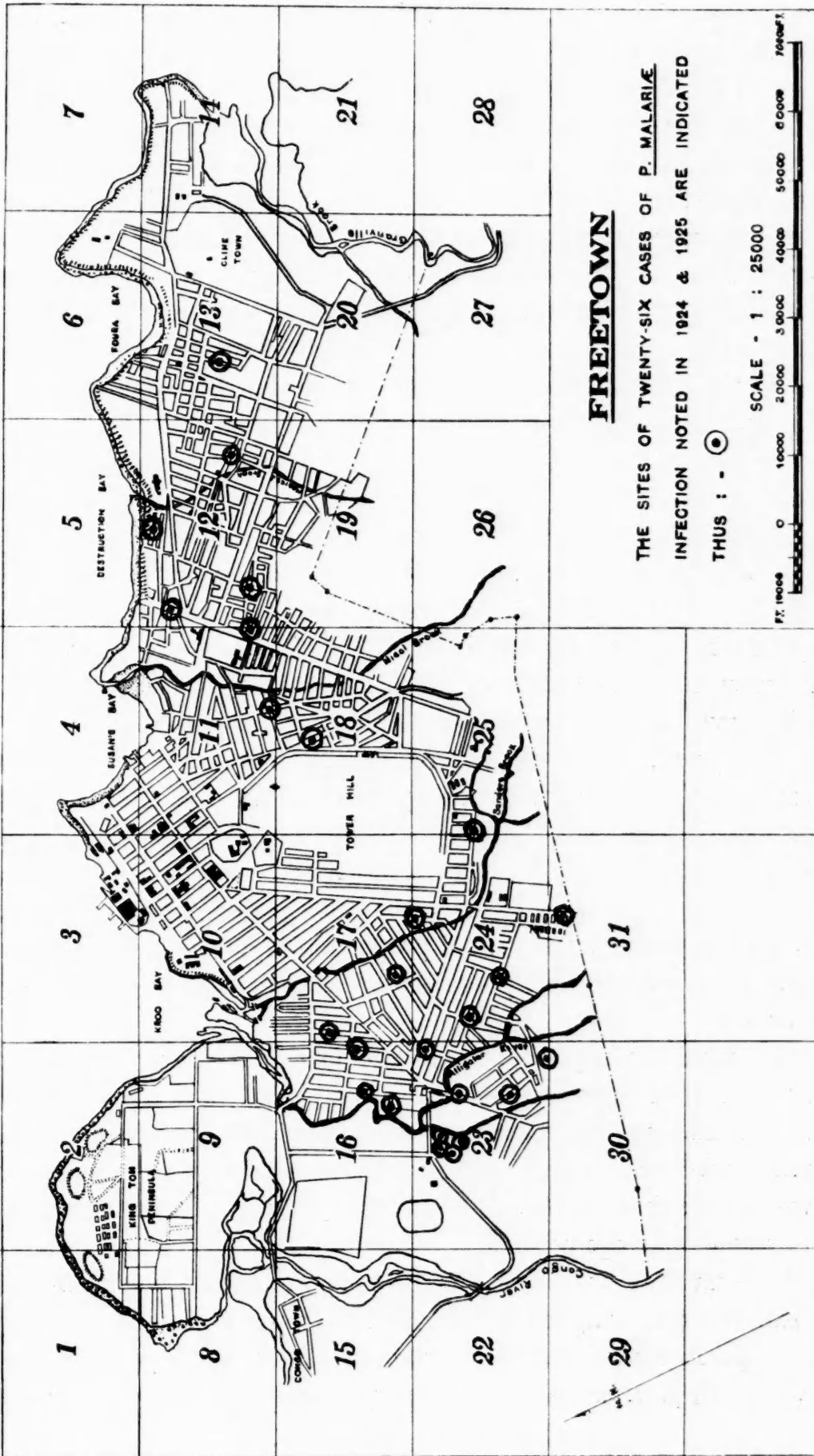
Comparing the distribution and proportions of *P. falciparum* and *P. malariae* throughout Freetown amongst 830 native children examined in 1924-25, and amongst 1,219 native children examined in 1931.

The Map section entitled 'Rural' refers to areas outside the town boundary. The figures for 1924-25 are given in italics, and those for 1931 in ordinary type.

Map section	Total examined	Total positive	Total infections		Total examined	Percentage positive	Percentage <i>P. falciparum</i> amongst positives	Percentage <i>P. malariae</i> amongst positives
			<i>P. falciparum</i>	<i>P. malariae</i>				
15	<i>5</i> 44	<i>2</i> 25	<i>2</i> 11	<i>0</i> 18				
2	<i>9</i>	<i>3</i>	<i>2</i>	<i>1</i>				
9	<i>2</i> 12	<i>1</i> 7	<i>1</i> 1	<i>0</i> 7				
16	<i>101</i> 104	<i>34</i> 36	<i>31</i> 20	<i>3</i> 16				
23	<i>40</i> 50	<i>18</i> 28	<i>9</i> 14	<i>8</i> 16	496	29.8	85.1	12.2
3	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>	591	42.8	49.8*	55.3*
10	<i>70</i> 69	<i>15</i> 27	<i>15</i> 19	<i>0</i> 9				
17	<i>188</i> 176	<i>49</i> 65	<i>44</i> 27	<i>3</i> 41				
24	<i>90</i> 126	<i>29</i> 62	<i>24</i> 32	<i>4</i> 32				
4	<i>10</i> 10	<i>0</i> 2	<i>0</i> 0	<i>0</i> 2				
11	<i>79</i> 119	<i>23</i> 34	<i>21</i> 21	<i>1</i> 14				
18	<i>76</i> 98	<i>23</i> 32	<i>22</i> 22	<i>1</i> 10				
25	<i>26</i> 35	<i>9</i> 9	<i>8</i> 7	<i>1</i> 4				
12	<i>101</i> 162	<i>37</i> 66	<i>32</i> 36	<i>5</i> 36	334	31.4	90.5	8.6
19	<i>9</i>	<i>4</i>	<i>1</i>	<i>3</i>	589	35.3	59.1	44.7
6	<i>3</i> 12	<i>1</i> 5	<i>1</i> 5	<i>0</i> 0				
13	<i>31</i> 112	<i>12</i> 46	<i>11</i> 24	<i>1</i> 21				
20	<i>3</i> 1	<i>0</i> 0	<i>0</i> 0	<i>0</i> 0				
14	<i>5</i> 31	<i>0</i> 10	<i>0</i> 7	<i>0</i> 3				
Rural ...	<i>58</i> 39	<i>27</i> 13	<i>19</i> 10	<i>2</i> 5	<i>58</i> 39	<i>46.5</i> 33.3	<i>70.4</i> 76.9	<i>7.4</i> 38.5

* The fact that the percentages of *P. falciparum* and *P. malariae* amongst the cases found positive exceed in some instances 100, is due to the occurrence of mixed infections.

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two groups showed a considerable difference, 41 per cent. being infected in the endemic area and 72 per cent. in the hyperendemic. A graph constructed on the average of these two figures would not be a true representation of the age incidence of malaria in Freetown, for this highly infected area only represents a small fraction (about one-twentieth) of Freetown, although one-third of Macdonald's examinations were conducted there. In addition his observations were conducted during July to March, a period when the malaria incidence is at its highest. Therefore, in making use of Macdonald's figures to continue the age incidence graph of Blacklock and Gordon (1925), we have left out of consideration the hyperendemic area, and have only made use of his figures for the endemic area.

In 1931 we examined the age incidence of malaria amongst 1,222 natives whose ages varied up to 20 years. The results are shown below in Graph I.

It will be seen that the graphs correspond very closely. It is unfortunate that Macdonald's 1926 observations do not extend beyond the age of 13, as it would be of interest to see whether the curve then descended sharply as in our 1931 observations. In both curves the incidence of malaria rises very sharply up to the age of 2 years, and from there on remains more or less consistently at the same high level until the age of 13 in the case of Macdonald's figures, and until the age of 10 in our observations, from which period it falls steadily to a level of 14 per cent. at the age of 16 years. Our figures for ages after this period are very scanty, but probably only a very small proportion of adults are to be found positive by ordinary peripheral blood examination after the age of 19 or 20.

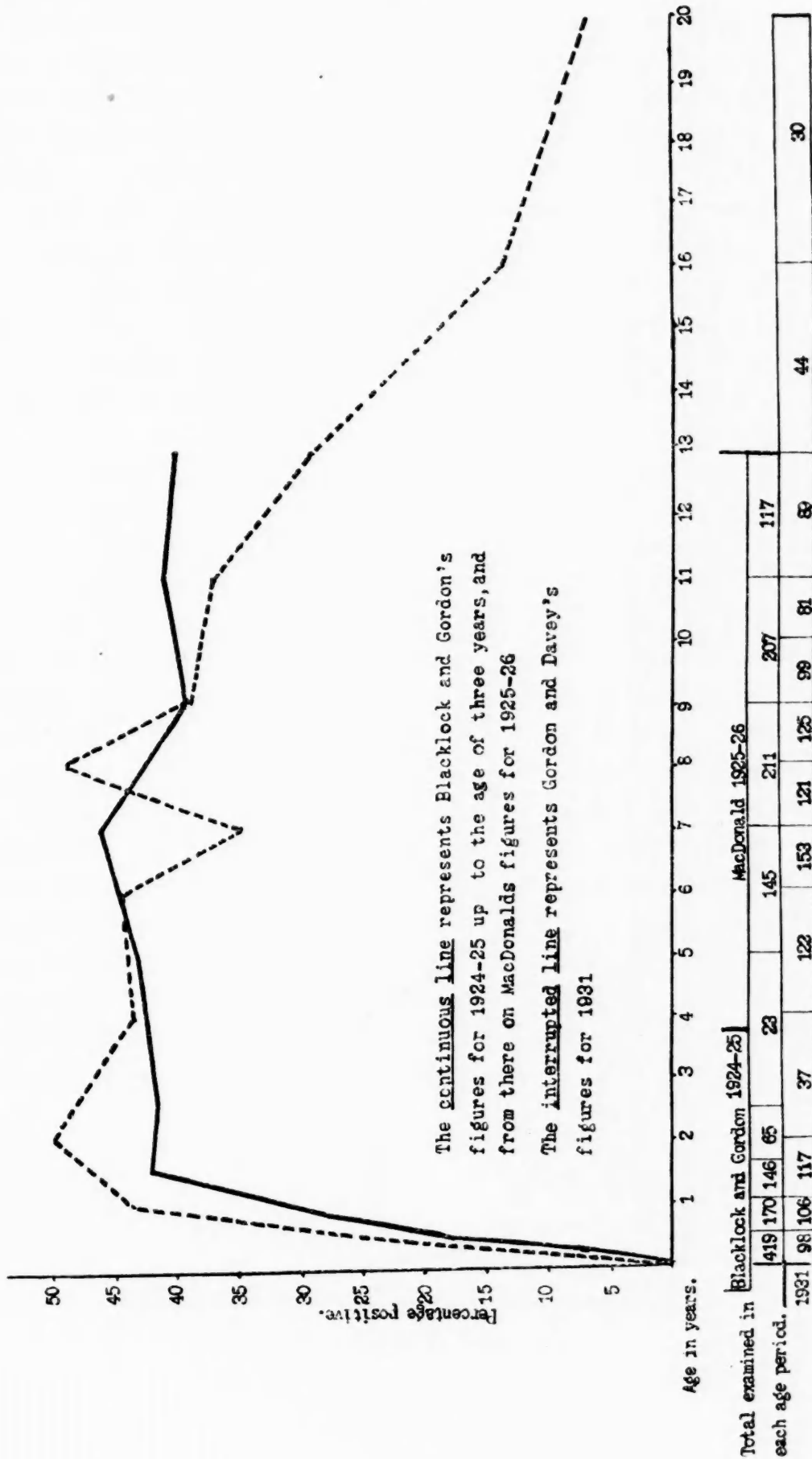
We have already referred to the fact that there has been no marked rise of malaria incidence in 1931 and that the increase of quartan malaria has occurred at the expense of the malignant tertian infection. The 1931 curve by its tendency to maintain the same level as that for 1924-26 shows that, in spite of some fluctuation, this is true of all age periods.

In Graph II we show the proportions of *P. falciparum* and *P. malariae* occurring in the different age groups during the year 1931.

Graph II shows in a very striking manner that, up to the age of 4 years, *P. falciparum* is still the predominant infection amongst

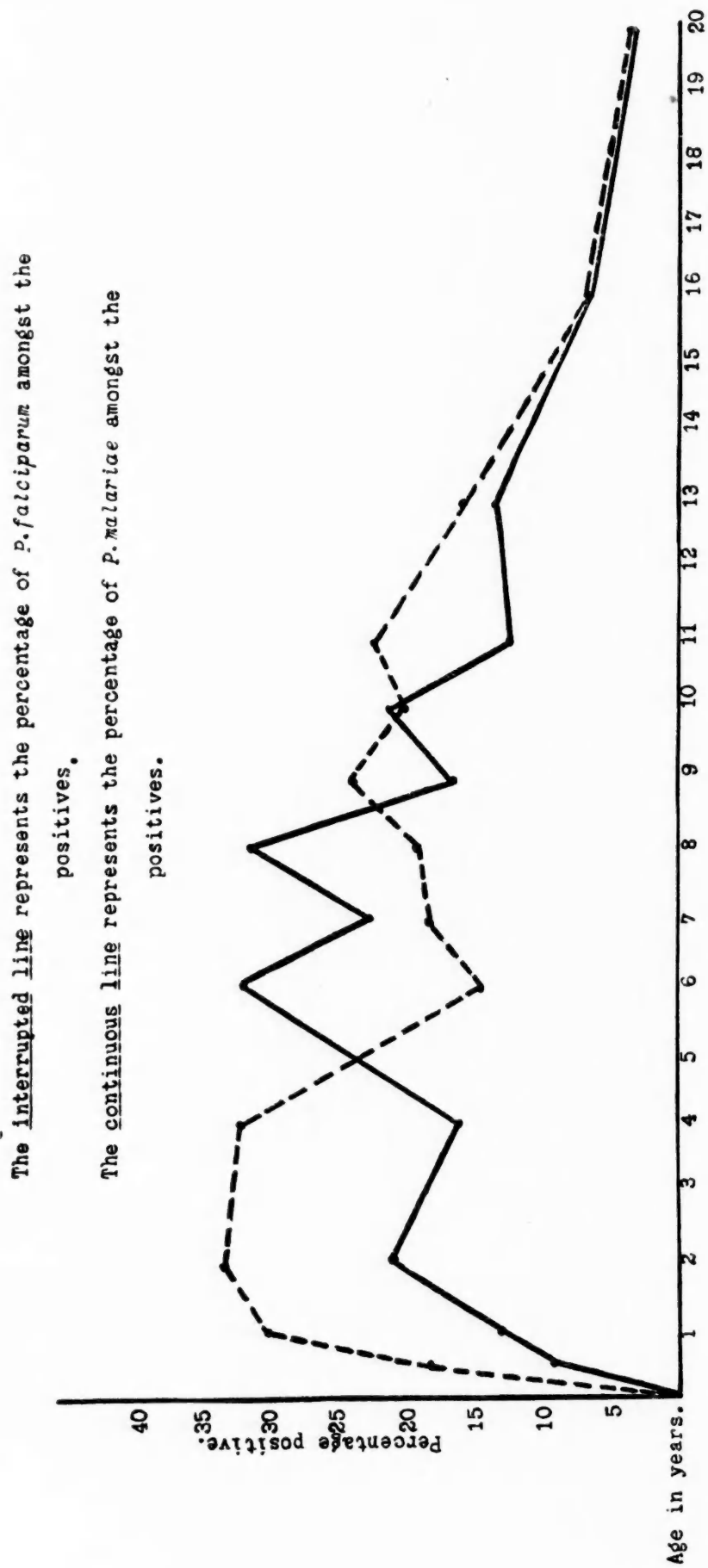
GRAPH I

Contrasting the age incidence of malaria in 1924-25 as given by Blacklock and Gordon, and in 1925-26 by Macdonald, with that obtained in 1931.



GRAPH II

Showing the age incidence of *P. falciparum* and *P. malariae* amongst 1,222 natives examined in 1931.



the native children of Freetown. From 5 to 8 years, *P. malariae* infections are in excess of *P. falciparum*, and from 9 years onwards the two infections are about equally distributed. We can give no explanation of the curious fact that in Freetown native children of 5 to 9 years show a higher incidence of quartan malaria than children in other age periods. Vialatte and Flye Sainte-Marie (1931) state that in Morocco quartan malaria, which is present amongst the indigenous population, did not affect the military garrison in which *P. falciparum* infections were common. These authors suggest that the prophylactic quinine taken by the troops was sufficient to protect them against *P. malariae* but not against *P. falciparum*. This theory does not appear to explain the higher incidence of quartan malaria in Freetown amongst the school children as compared with the infants up to 3 years of age, as neither group was receiving prophylactic quinine. Similarly, some of the Europeans already mentioned who contracted quartan malaria were taking prophylactic quinine.

A number of hypotheses suggest themselves dependent on the one hand on the human host, and on the other on the insect vector. Amongst the latter it appears possible that only a very small proportion of the anophelines become infected with quartan malaria, so that the 'exposure risk' of the child increases with increasing age, becomes maximum at the 5 to 9 age period. The fall in the infection rate after this age probably depends on increasing immunity. Dr. G. Macdonald has suggested to us that both factors may be concerned. He postulates, as we do, that fewer anophelines are infected with *P. malariae* than with *P. falciparum*, and he explains the maintenance of the high quartan rate amongst the children on the supposition that *P. malariae*, when once acquired, persists longer in their circulation than does *P. falciparum*. In Section VI we discuss the question of the insect vector and the great difficulty of infecting anophelines with *P. malariae*.

VI. THE INSECT VECTOR OF *P. MALARIAE*

James (1931) has drawn attention to the great difficulty of producing infection of the salivary glands of anophelines with *P. malariae*, and cites various instances, besides his own series, of failure successfully to produce infection. During the last two years

we have at various times endeavoured to infect anophelines by feeding them on patients whose blood contained numerous quartan gametocytes. Our constant failure to do so induced us to consult as many references as possible to feeding experiments with this parasite, in which search Covell's (1927 and 1931) publications have proved most helpful.

As a result of this search we were able to compile a list of some 1,500 anophelines, comprising some twenty-five species, which had been fed by various observers on cases of *P. malariae*. Unfortunately a large proportion of these experiments must be omitted from consideration because they do not contain sufficient data to allow of any conclusions being drawn from them. In assessing the value of any experiments claiming to have positive results we have adopted the following criteria: (1) The mosquitoes used must have been laboratory bred, or else obtained in a district free from any suspicion of malaria (as, for example, experimental transmissions conducted in England); (2) Sporozoite infection of the salivary glands must have been obtained; oocyst formation in the stomach is not necessarily a proof that the species is capable of transmitting malaria; (3) Where claims are made that a mosquito is capable of being infected with a particular species of *Plasmodium* it is important that great care should have been taken to exclude the possibility of a mixed infection in the host; the examination, however careful, of a single thin film is by no means sufficient to exclude such a contingency. The omission of standards (1) and (3) need not invalidate a negative result, but the omission of standard (2) frequently does. As a result of the application of these criteria we have omitted the experiments of some twelve observers who claimed to have proved certain species of anophelines as transmitters of *P. malariae*. Thus, Ross, Annett and Austen (1900) are cited as having infected both *A. costalis* (*A. gambiae*) and *A. funestus* with quartan malaria. On consulting the originals it will be seen that they fed five 'wild' *A. funestus* on a quartan carrier, of which two were subsequently dissected and one found infected with zygotes in the stomach. In the case of *A. costalis*, they fed one 'wild' specimen on a quartan carrier, and found on dissection two days later that it was infected with zygotes. To quote a more modern example, Hylkema (1920) fed 117 *A. ludlowi* on a case of quartan malaria and dissected

them seven days later, at which date twenty-five of the mosquitoes were found infected with oocysts.* It is obvious that it is impossible to state from such data, (1) that *A. ludlowi* is a transmitter of *P. malariae*, because the oocysts might not have progressed to sporozoite infection of the glands; (2) it is equally impossible to state that *A. ludlowi* is not a transmitter, as the oocysts might have completed their cycle.

For the same reason the claims of Swellengrebel and others (1919) and of Kinoshita (1906) that they have proved that *A. hyrcanus* is capable of transmitting quartan malaria must be disallowed. The former authors fed 227 wild *A. hyrcanas*, obtained from places known to be 'almost free from malaria,' on cases of *P. malariae* who had numerous gametes in their blood, some of which showed exflagellation at the time of biting. At the subsequent dissection only the guts were examined, and of these two (0.8 per cent.) were found to contain oocysts. It is of interest to compare this result with that obtained by the same authors when feeding this species on cases of *P. falciparum* and *P. vivax*. Of 378 *A. hyrcanus* fed on a case of *P. falciparum*, fifteen (3.9 per cent.) subsequently exhibited oocysts at dissection. Of 248 fed on cases of *P. vivax*, sixty-four (26 per cent.) became infected with oocysts. Kinoshita (1906), in his experiments, also used wild mosquitoes (*A. hyrcanus* and *A. listoni*) and only recorded the results of the gut dissections. In Table V we have briefly summarised the available literature admissible under the criteria mentioned above, and in addition we have included a statement as to whether the species in question has ever been experimentally infected with *P. falciparum* and/or *P. vivax*.

It can be seen from the above table that the only successful records quoted are those of Stephens and Christophers (1902), who succeeded in producing infection of the salivary glands in two out of six *A. culicifacies*, fed on four cases of *P. malariae* in India. It is of interest to note that the authors refer to the infection as 'sporozoites scanty,' and it must be remembered that apparently the examination of the patient's blood in these early infection experiments did not include the use of thick films, so that in accepting

* Hylkema, with a few exceptions, used 'wild' *A. ludlowi* in his experiments, the captured anophelines being kept for a couple of days before being allowed to feed on the patient. By this means Hylkema was of the opinion that it was possible to distinguish natural oocyst-infections from experimental infections, when the insects were subsequently dissected.

TABLE V

Showing the results of various observers' attempts to infect anophelines with *P. malariae*.

Species	Authority and date	No. of fed anophelines dissected	No. of anophelines infected in the glands. Negatives include dissections of the stomach within the period of time during which oocysts might be expected	Experimental infections in the glands with	
				M.T.	B.T.
<i>A. albotacniatus</i> ...	Swellengrebel <i>et al.</i> 1919	1	○	○	—*
<i>A. barbirostris</i> ...	Walker and Barber. 1914	13	○	○	○
	Swellengrebel <i>et al.</i> 1919	1	○		
<i>A. crucians</i> ...	Beyer <i>et al.</i> 1901. ...	3	○	+	+
	Root. 1924 ...	3	○		
<i>A. culicifacies</i> ...	Stephens and Christophers. 1902	6	2	—	○
<i>A. fuliginosus</i> ...	Stephens and Christophers. 1902	6	○	○	—
<i>A. hyrcanus</i> ...	Carter. 1927 ...	15	○	—	—
<i>A. kochi</i> ...	Swellengrebel <i>et al.</i> 1919	4	○	○	○
<i>A. leucosphyrus</i> ...	Swellengrebel <i>et al.</i> ...	2	○	—	○
<i>A. listoni</i> ...	Carter. 1927 ...	2	○	—	○
	Kinoshita. 1906 ...	2	○		
<i>A. ludlowi</i> ...	Swellengrebel. 1919 ...	18	○	+	+
<i>A. maculipennis</i> ...	Vestschezerow. 1926 ...	9	○	+	+
	James. 1931 ...	305	○		
<i>A. maculatus</i> ...	Green. 1929 ...	160	○	+	+
<i>A. minimus</i> ...	Walker and Barber. 1914	40	○	+	○
<i>A. pseudopunctipennis</i>	Darling. 1910 ...	1	○	—	—
	Davis and Shannon. 1928	3	○		
<i>A. punctimacula</i> ...	Darling. 1910 ...	1	○	○	—
<i>A. quadrimaculatus</i>	Beyer <i>et al.</i> 1901 ...	5	○	+	+
<i>A. rhodesiensis</i> ...	Gordon and Macdonald. 1930	7	○	+	—
<i>A. rondoni</i> ...	Davis and Shannon. 1928	9	○	○	○
<i>A. separatus</i> ...	Pendlebury. 1926 ...	8	○	○	○
<i>A. stephansi</i> ...	Stephens and Christophers. 1902	4	○	+	+
<i>A. subpictus</i> ...	Stephens and Christophers. 1902	3	○		
	Walker and Barber. 1914	21	○	+	+
	Carter. 1927 ...	7	○		
<i>A. tarsimaculatus</i> ...	Davis and Shannon. 1928	1	○	—	—
	Darling. 1910 ...	1	○		
<i>A. tessellatus</i> ...	Swellengrebel <i>et al.</i> 1919	5	○	—	○
<i>A. umbrosus</i> ...	Swellengrebel <i>et al.</i> 1919	32	○	+	○
<i>A. vagus</i> ...	Swellengrebel <i>et al.</i> 1919	10	○	○	○
	Carter. 1927 ...	2	○		

* The sign — means that we could find no records of experiments, or else that the number of mosquitoes tried with negative results was less than five.

these results the possibility of a mixed infection must be borne in mind. To us it seems a very surprising fact that the evidence regarding the transmission of *P. malariae* by anophelines should rest on such a small basis of fact.

In another paper (Gordon *et al*, not yet published) we describe a detailed anopheline survey of a portion of Freetown carried out during 1930-31. This survey chiefly covered the area included in Section 17 in the attached map. This area, as can be seen from Table IV, showed a proportion of quartan malaria which was among the highest obtained throughout the town. Only two species of anophelines were found in the houses examined, *A. costalis* (*A. gambiae*) and *A. funestus*, the latter being so very rare as to be negligible. Our outdoor experiments have also confirmed the fact that these two anophelines are the only species likely to feed on human hosts in Freetown. It appears certain, therefore, that if *P. malariae* is transmitted by an anopheline, it must be by *A. costalis* alone, or else by *A. costalis* and to a much less extent by *A. funestus*. Although we have successfully infected both these anophelines with *P. falciparum*, we have completely failed to do so with *P. malariae*. Our results are shown below in Table VI.

It will be noted that we have not stated the number of gametocytes present except in certain instances. In the case of experiments conducted with *P. falciparum* we always calculated the number of crescents by means of a thick film, but in the case of *P. malariae* a gametocyte estimation, in our opinion, cannot be made accurately except by a count made on thin films, which involves an amount of labour out of proportion to the value of the information gained. We therefore confined ourselves to selecting the most suitable cases from amongst the quartan carriers at our disposal, and to making sure by means of thick and thin films that they contained a sufficient number of gametocytes at the time of feeding. As regards exflagellation, this appeared to be of almost constant occurrence in the cases selected by us, in distinction to *P. falciparum* which seems to vary with different individuals and at different times in the same individual.

TABLE VI

Showing the results of attempts to infect *A. costalis* (*A. gambiae*) and *A. funestus* with *P. malariae*.

Patient	Gametocytes	Species of anopheline	No. less than full fed	No. full fed	No. dissected after feeding		Result
					Over 3 and under 10 days	Over 10 days	
B.A.	++	<i>A. funestus</i> ...	5	7	4	8	○
A.L.	+	<i>A. funestus</i>	2	...	2	○
D.B.	++	<i>A. funestus</i> ...	1	2	...	3	○
S.C.	++	<i>A. costalis</i>	1	...	1	○
S.F.	++(+)	<i>A. costalis</i> ...	4	2	1	2	○
S.F.	++(+)	<i>A. costalis</i> ...	3	4	3	3	○
S.F.	++	<i>A. costalis</i> ...	6	4	4*	6	○
E.T.	+++ 20 gametocytes to 1,000 leucocytes	<i>A. funestus</i> ...	1	1	○
E.T.	+++ 20 gametocytes to 1,000 leucocytes	<i>A. funestus</i> ...	5	3	○
E.T.	+++ 20 gametocytes to 1,000 leucocytes	<i>A. costalis</i> ...	5	1	1	4	○
I.C.	+ 8 gametocytes to 1,000 leucocytes	<i>A. costalis</i> ...	8	1	3	5	○
I.C.		<i>A. costalis</i> ...	3	4	3	3	○
M.H.	+	<i>A. funestus</i>	1	...	1	○
M.H.	+	<i>A. costalis</i> ...	4	1	...	5	○

* In one of these mosquitoes dissected on the fourth day two zygotes were found, the only instance in which we found oocyst formation in this series.

ADDENDUM

The recent marked rise in the incidence of quartan malaria in Freetown appears to show no sign of abatement. This is shown by the following figures compiled during the three months following the completion of our paper in October, 1931. Two hundred native children, aged from three to fourteen years, have been examined for the first time; of these, one hundred and five (52.5 per cent.) have been found infected. The distribution of the infections was as follows: *P. falciparum*, 50.8 per cent.; *P. malariae*, 68.6 per cent.; *P. vivax*, 1.9 per cent.

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NOTES ON AFRICAN MOSQUITOES

BY
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I. THE PUPAE OF SOME AFRICAN ANOPHELES

The material dealt with in this section has been mainly supplied to the writer through the kindness of Mr. G. H. E. Hopkins, Entomologist, Uganda, and Dr. M. A. Barber of the International Health Division of the Rockefeller Foundation.

The terminology followed is that originated by Macfie (1919) with most of the modifications introduced by Senevet (1930), slight alterations being made for the sake of convenience. In the descriptions the number of branches of the bristles and hairs is given, and it is noted whether they are particularly long or well developed or very short; when no reference is made to the size, the hair is of moderate or rather small size.

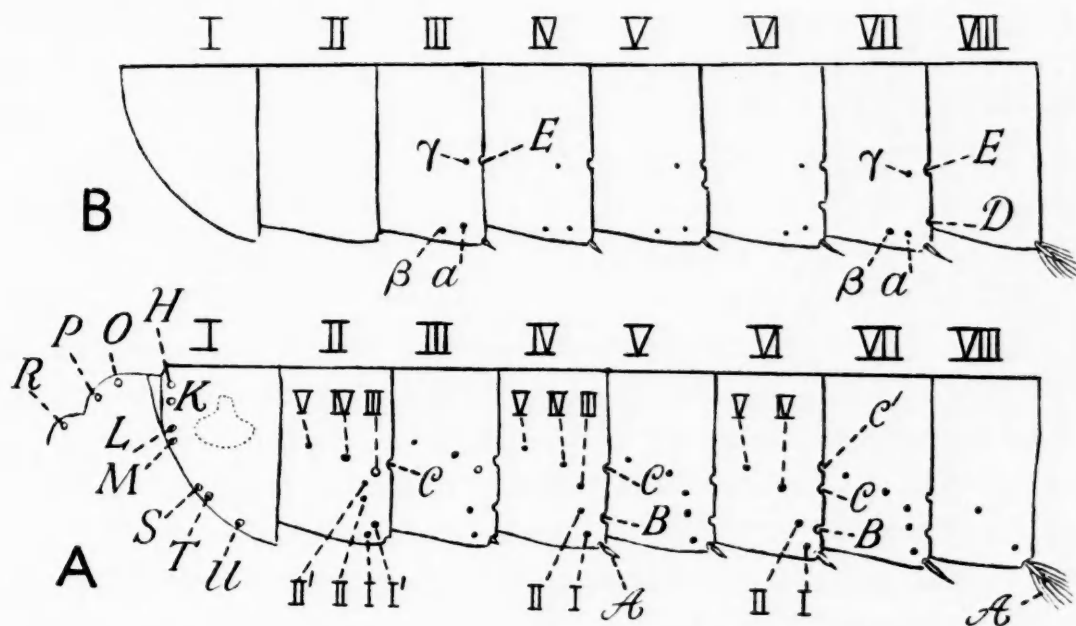


FIG. 1. Diagrams illustrating nomenclature of metathoracic and abdominal chaetotaxy. A.—dorsal; B.—ventral. After Senevet, somewhat modified.

Variability in pupal chaetotaxy. Owing to the extreme variability exhibited by the branching of many of the bristles, in some of the following species it should be noted that the figures given probably only indicate the approximate limits of variation. In this connection it seems well to point out that detailed descriptions based on a study of only one or two examples of a species may have to be modified considerably when further material is examined. Thus, while in many cases the pupal differences described by Senevet (1930 and 1931) between closely allied species will probably be found to be of great value in separating these forms, it would perhaps be safer to wait until further examples have been examined before regarding his conclusions as final. A description of any two examples of *A. obscurus* (see below), would be found inapplicable to other specimens in many details of the chaetotaxy.

ANOPHELES OBSCURUS Grünb.

PUPA (fig. 2). The respiratory trumpet is asymmetrical. It is widest in a direction almost at right angles to the long axis of the pupa, relatively shallow and deeply cleft above and below to form two unequal portions. At one side of the dorsal cleft the wall is produced into a prominent process (*d.p.*), the opposite side appearing as a very much smaller lobe (fig. 2, B and C, *lo.*). Ventrally the two sides divided by the cleft are equal in size, though they may appear otherwise owing to folding when the specimen is mounted flat. The specimen from which fig. 2, C, was made was not mounted but floating in fluid in a hollow slide. The actual respiratory opening (*o*) lies between the dorsal cleft and the edge of the lining of the trumpet, which is incomplete dorsally.

Chaetotaxy. Metathorax. *R*, long and bifid; *P*, rather long and 4- or 5-branched; *O*, shorter and simple or bifid. *Abdomen*, dorsal (fig. 2, A), showing considerable variation; *A*, as usual, spine-like on third to seventh segments and reduced to a minute tubercle on second. In West African specimens the bristles are relatively very short on the fourth to seventh segments (as on left side of fig. 2, A), but in East African specimens examined they were more normal. *A*, on eighth segment may be almost simple or with the margin produced into fine processes variable in number, position and

length, as shown in fig. 2, A ; this bristle may also show indications of very short processes on the sixth to seventh segments. C, on sixth and seventh segments represented by tufts composed of a very variable number of bristles, often greatly unequal in length and usually darkly pigmented. When the bristles are numerous these tufts appear dense and blackish in colour ; the appearance varies

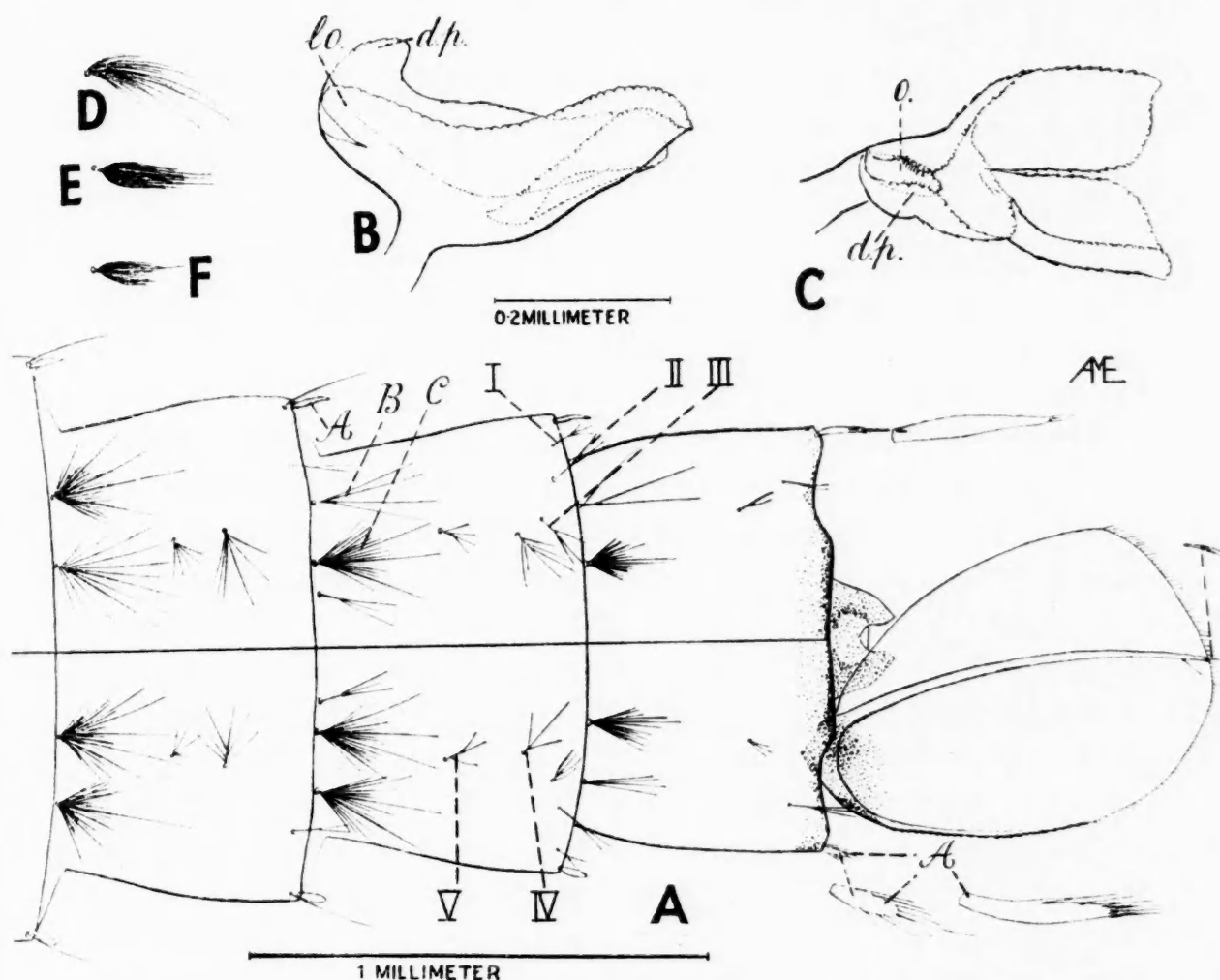


FIG. 2. *Anopheles obscurus*, pupa. A.—last few segments of abdomen, dorsal aspect, showing variability of bristles and their appearance as seen in water or carbol alcohol. B.—respiratory trumpet, lateral view ; C.—the same viewed from above. D, E, and F.—tufted bristles from fifth to seventh segments, as seen in specimens mounted in canada balsam or glycerine jelly. d.p.—dorsal process ; lo.—small dorsal lobe ; o.—opening. Other lettering as in Fig. 1.

considerably according to the medium in which the pupa is examined (compare in fig. 2, A and D to F). C is well developed and branched (8 to 18) on the third and fourth segments and usually on the fifth, but it may be a dense tuft on this segment and, in one specimen examined, the two sides were dissimilar in this respect. On the

second segment this bristle is variable in size and number of branches (7 to 18) ; *C'*, on sixth segment with about 2 to 3 branches. *B*, with about 10 to 14 branches on the third segment ; on fourth and fifth usually and sixth occasionally, they are dense, pigmented tufts ; on the seventh segment and usually on the sixth they have 2 to 5 equal branches arising from a short stem. Branching of bristles of first abdominal segment very variable. Many of smaller hairs also variable. *Dorsal*, *I*, 2- to 5-branched on second segment, simple on most of the others but may be bifid on third and seventh. *I'*, 2- or 3-branched (second segment only). *II*, 4- to 8-branched on second segment and on third to seventh segments branched as follows :—*I* to 4 ; 3 to 4 ; 2 to 3 ; *I* to 3 ; *I* to 2. *II'*, 2- to 4-branched (second segment). *III* on segments two to five respectively :—2 to 3 ; 7 to 10 ; 5 to 9 ; 2- to 4-branched and, on seventh segment, 3 to 5. *IV*, about 6- to 10-branched on second to fifth segments ; 5- to 7- on sixth and 3- to 9- on seventh. *V*, usually relatively large on second to fifth segments, branching very variable. On eighth segment *VII* is 2- to 4-branched and *VIII* minute and usually split into 2 or 3. *Ventral*, *D*, 2- to 4-branched on seventh, simple or bifid on sixth segment ; *E*, about 2- to 4-branched on third and fourth segments, simple or bifid on fifth and sixth, 2- to 3-branched on seventh. *a*, 2- to 4-branched on third to sixth segments and 3- to 6- on seventh. *β*, about 2- to 4-branched where present. *γ*, simple or bifid on third to fifth segments, simple to 4-branched on sixth and 2- to 3-branched on the seventh.

Paddles with fringe round distal margin abruptly replaced externally by short spines. Apical bristle short and either simple or split or produced into a variable number of processes, usually toward the apex. Sub-apical hair 2- or 3-branched.

Described from a number of pupal pelts taken near Lagos, 1930-31, and the Firestone Plantations, Liberia, 1931, by Dr. M. A. Barber, also four pupal pelts taken near Kampala, Uganda, 24.xi.1929, and two others from East Africa, the last six from Mr. G. H. E. Hopkins.

This pupa differs from that of *mauritanus* in the shape of the trumpet which, in that species does not show the marked asymmetry of *obscurus* and, further, the trumpet appears deeper in lateral view and its margins are fluted in *mauritanus*. *A* is apparently always normally developed on the eighth segment in *A. mauritanus*.

C on the sixth and seventh segments as far as our material of *mauritanus* (vars.) and existing descriptions show is either simple or bifid in that species, not tufted as in *obscurus*. *A. umbrosus*, as described by Senevet from a single pelt, differs in this character, *C* being trifid on the sixth and simple on the seventh segment. According to Dr. Senevet's figures the trumpet is asymmetrical in a number of species of *Anopheles*, s.s.

ANOPHELES KINGI Christophers

PUPA (fig. 3). Respiratory trumpet rather broader than in many species; lateral margins convex; opening continued almost to the base.

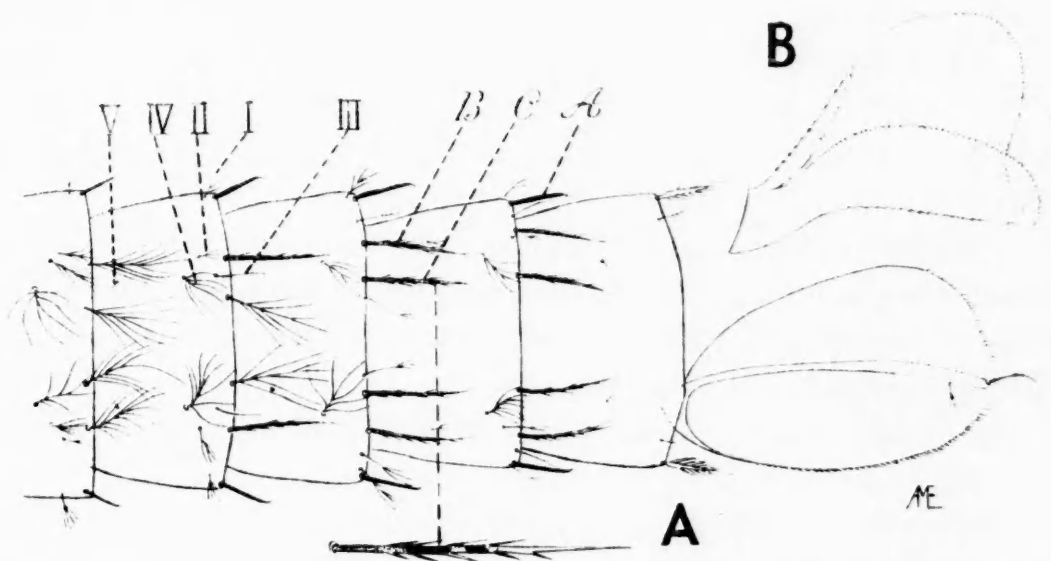


FIG. 3. *Anopheles kingi*, pupa. A.—last five segments of abdomen, dorsal aspect. B.—respiratory trumpet. Other lettering as in Fig. 1. Scale as in Fig. 4.

Chaetotaxy. Metathorax. *R*, long, branched (8 to 9); *P* and *O*, shorter and curved, about 6 branches. *Abdomen, Dorsal.* *A*, long, slender and pointed on fourth to seventh segments; greatly reduced as usual in second and third segments and on eighth normally feathered. *B*, on the fifth to seventh segments these are stout, spine-like bristles about two-thirds the length of the succeeding segment and bear short, stiff, lateral spinous processes, which are more numerous on the outer side; the shaft shows irregular bands of black pigment. On the third and fourth segments, bristles *B* are branched with 11 to 12 and 9 to 11 branches. *C*, on the sixth and

seventh segments similar to *B*, except that the lateral barbs tend to be equally numerous internally; on second to fifth segments these are long bristles with about 6 to 9 branches. *C'*, on sixth segment is a hair, bifid or trifid towards the apex. Other bristles and hairs on second to seventh segments branched as follows:—I, 5 to 7; 5 to 7; 3 to 4; 2 to 6; 3 to 6; 1 or 2 to 3 or 4. II, 7 to 10; 4 to 6; 2 to 4 (small); 1 to 4; 1 to 3; 1 to 3. III, 1 to 2, 1 to 3; 4 to 7; simple; absent; 1 to 5. IV, size variable, but usually rather large except on seventh segment, 8 to 10; 7 to 11; 5 to 7; 6 to 8; 5 to 7; 5 to 7. V, minute, simple or bifid on second to sixth segments; may be trifid on seventh. *First Segment*. *H*, 1; *K*, 4 to 6; *L*, delicate, 7 to 9; *M*, long, well developed, 4; *S*, 4 to 5; *I*, fairly long, 3 to 5; *U*, shorter, simple. *Eighth segment*. VII, 2 to 4. VIII, minute simple or bifid. *Ventral*, third to seventh segments: *D*, where present, apparently 3- to 4-branched. *E*, usually 3- to 4-branched. *a*, on third to seventh segments respectively, 3; 3 to 4; 3 to 4; 2 to 3; 3 to 5. γ , simple except on seventh, where about 3- to 8-branched. β , where present, about 1 to 4.

Paddles rather elongated (see fig. 3). Fringe with fine hairs of distal margin abruptly replaced externally by coarser, but finely-pointed processes. Apical bristle curved as shown in the figure, sub-apical hair trifid in its outer half.

Described from six pupal pelts; Mount Elgon, 1930, received from Mr. Hopkins.

This pupa is unique among those of *Anopheles* so far described in the characters of the bristles *B* and *C* on the fifth to seventh segments. As Dr. Senevet's (1930-1931) account of the pupae shows, these may be either simple, branched or tufted; in no other species have they been shown to bear short stiff lateral processes as in the present species. It is interesting to see that the pupa of other species of *Neomyzomyia* group, *punctulatus* as described by Senevet and also *nili*, *smithii* and *cinctus* (see below) are quite different from *A. kingi* in this respect.

Pupal pelts of *A. ardensis*, collected by Mr. E. G. Gibbins in Uganda and recently received from Mr. G. E. H. Hopkins, showed a distinct approach to *A. kingi* in the character of these bristles. The lateral processes were, however, much longer and finer than in *kingi*.

ANOPHELES GARNHAMI Edw.

PUPA (fig. 4). Respiratory trumpet shaped as shown in figure ; opening continued almost to the base.

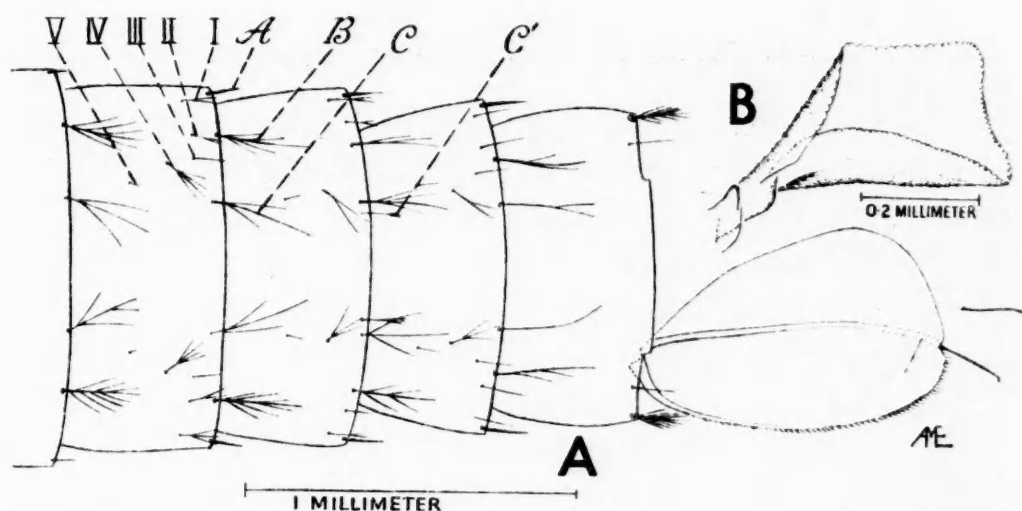


FIG. 4. *Anopheles garnhami*, pupa. A.—last few segments of abdomen, dorsal aspect ; B.—respiratory trumpet. Lettering as in Fig. 1.

Chaetotaxy. Metathorax. *R*, long and simple ; *P*, shorter and 4-branched distally ; *O*, long and simple. *Abdomen, Dorsal* : *A*, a slender pointed spine on segments five to seven, length as shown in figure ; bilateral branches normally developed on eighth segment. *B*, with about 7 unequal branches on third segment ; well developed on fourth to seventh and with about 6 to 9 ; 5 to 7 ; 5 or 6 ; and 3 branches on these segments respectively. *C*, fairly well developed and with 5 or 6 branches on second segment ; well developed on following segments and with about 6 or 7 ; 3 to 5 ; 3 or 4 ; 3 to 5 branches on third to sixth respectively ; on seventh segment simple or bifid. *C'*, on sixth segment with extremity trifold. First segment : the usual large dentritic hair ; *H*, simple ; *K*, 4- to 6-branched ; *L*, 4 to 6 ; *M*, long, simple or bifid ; *S*, 3-branched ; *I*, long, bifid or trifold ; *U*, apparently bifid or trifold. Shorter hairs : on fifth to seventh segments, as shown in the illustration, but branching probably more variable than shown by the two specimens available. Owing to the probability of variation the other small hairs are not described.

Paddles. Shaped as shown in figure. Fringe present only on outer side of the apical bristle ; its processes rather coarse and

tooth-like at about the middle of the margin but becoming gradually finer distally. In both pelts the apical bristle is almost straight.

Described from the pelts of two pupae, Sipi, Mount Elgon, Mr. Hargreaves; received from Mr. Hopkins.

ANOPHELES FUNESTUS Giles. Type form

The pupa of this species was described by Senevet (1930) from three pelts of specimens taken in the Gold Coast. A study of the pelts of twelve authentic *funestus* from Sierra Leone, S. Nigeria, Belgian Congo and Uganda shows that, while the figures given by Senevet for the branching of the larger bristles seem to represent the usual condition, there is a considerable amount of variation. This concerns even the branching of bristles *B*, *C* and *C'* on the fifth to seventh segments, and is sometimes such as to invalidate the key given by Senevet. *C* may be simple on either the sixth or seventh, so that the distinction from the pupa of *A. listoni* (as described by Senevet) is slightly less marked than would appear. In this connection it should be noted, however, that in all *funestus* pelts examined the relative length of the spine-like bristle *A*, and also of *C*, on the fifth to seventh segments was about as shown by Senevet and considerably shorter than in *listoni* or the recently described *A. funestus* subsp. *leesoni* (Evans, 1931b). It should be noted that, while the spines and fringe bordering the paddles have the same character that Senevet describes, the relative length of these is considerably shorter in specimens I have examined than in the figure given by that author.

Abdominal chaetotaxy. Variations seen in the branching of the principal bristles. On segments three to seven respectively. *B*: 6 to 13; 4 to 7; 2 to 5; 3 to 5 (? 6); 2 to 4. *C*: 5 to 9; 3 to 7; 2 to 3; 1 to 2; 1 to 2. *C'* on sixth: simple or bifid.

II. MOSQUITOES COLLECTED IN LIBERIA BY DR. M. A. BARBER

In the course of his investigations on the Firestone Plantations, Liberia, in February and March, 1931, Dr. Barber made an extensive survey of the Culicidae and added eight species and one variety of *Anopheles* and nine culicines to the list of those recorded from Liberia (Bequaert, 1930). Valuable material of the species collected,

including bred specimens with their larval and pupal pelts, was very kindly submitted by Dr. Barber for study, and presented to the collections of this School.

Amongst a number of interesting discoveries made by Dr. Barber was that of the early stages and males and complete females of *Anopheles cinctus*, a species which has apparently never been re-discovered since one imperfect female was found in the Gold Coast about twenty years ago. A very distinct new variety of *A. obscurus* was found breeding in shaded hill streams on the Plantations and Dr. Barber succeeded in obtaining a female, with its larval and pupal pelts, of a species of the *marshalli* series, thereby giving the clue to a problem of several years' standing. Many valuable observations which Dr. Barber made on the anophelines of Liberia are recorded in the Report, which has been or is about to be published in the 'American Journal of Hygiene.' A full list of the mosquitoes collected during the survey appears in this Report, but it may be as well to make some comment here on those species which are new to the recorded fauna of Liberia. Only two species of *Anopheles*, *A. costalis* (*gambiae*) and *A. funestus* were listed in 1930, but Dr. Barber found in addition :—*A. nili* and *A. theileri* var. *hancocki* (some adults captured indoors) ; *A. mauritanus* var. *paludis* ; *A. obscurus* ; *A. obscurus* var. *nowlini*, n.var. ; *A. hargreavesi* ; *A. cinctus* ; *A. smithii* (one larva, the first record of this species away from the environs of Freetown) and *A. barberellus* n.sp. Culicini not recorded previously included *Culex guiarti* (rather a-typical) ; *C. inconspicuus* ; *C. nebulosa* ; *C. cinereus* ; two specimens with larvae somewhat intermediate in character between *C. cinereus* and *C. nebulosus* ; *Mansonia uniformis* ; *A. (Stegomyia) vittata* ; *Mimomyia mimomyia-formis* and *Uranotaenia balfouri*.

It is very interesting to record that among a collection of anophelines captured daily in a hospital at Bolahun in the interior of Liberia, August to September, 1930, by Dr. E. W. H. Maass, and kindly presented to this School, four species were represented, the numbers being as follows :—*A. costalis*, 533 ♀♀, 1 ♂ ; *A. funestus*, 45 ♀♀ ; *A. theileri* var. *hancocki* (some slightly a-typical in wing markings), 7 ♀♀ ; *A. nili*, 2 ♀♀.

ANOPHELES BARBERELLUS n.sp.*A. domicolus* Blacklock and Evans, 1926.

This small species is one of the *marshalli* series and has features in common with *A. domicolus* Edw. to which some examples have formerly been referred.

FEMALE. Palps 3-banded, the two distal bands about equal to each other and very broad, at least twice the intervening dark area in specimens I have seen. Proximal band very narrow. Relative lengths of third to fifth segments respectively as 53:27.8:13.2. Head with the usual white and dark upright forked scales. Antennae with several flat, white scales on first segment of the flagellum.

Thorax. Mesonotum rather thickly clothed with white or creamy narrow curved scales, those on about the posterior third, on the whole, much narrower than the rest; scales at the sides in front broad and truncated and extending backwards for a short distance as a series of flat overlapping scales above the fossae. A patch of very narrow, long, white scales may be present in front of the wing root. Integument mainly grey, that of fossae brown. Presternum apparently without a bristle. Scutellar bristles in most of specimens with outer half or less very dark, contrasting markedly with the paler proximal portion.

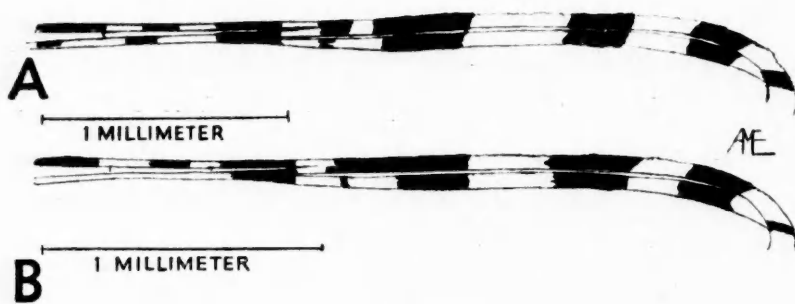


FIG. 5. Diagrams showing dark and light markings of costa and first vein. A.—*Anopheles barberellus*, n.sp., cotype ♀; B.—♂ specimen referred to this species.

Wings with dark areas of costal region sometimes considerably reduced. In the two bred specimens the second main dark area is completely interrupted on the costa (fig. 5 A). In other specimens this dark area may be completely interrupted on one wing and bridged across by dark scales on the other; bridged across on both wings,

or completely absent (two specimens provisionally referred to this species). The proximal division of this dark area is reduced in some specimens and, in one case, was only represented on the subcosta. The basal quarter of the costa shows two well-marked pale interruptions which may be much longer than the intervening dark area. The third and fourth main dark areas are somewhat variable in extent but tend to be shorter than the pale areas, preceding them. On the first vein, the first, third and fourth dark area equal to those on the costa and uninterrupted; the second always interrupted and the two portions usually shorter than those of the costal spot when interrupted. Second vein with a dark area of variable extent at or near the bases and apices of both branches; stem with two rather large dark areas. Third vein mainly pale scaled with a small dark spot at the apex and one or two basally. Fourth vein with two dark areas on upper and one on lower branch; stem with a long dark area near the fork, and a small spot usually present about mid-way between the fork and base. Fifth vein with a dark area at the fork, involving both branches and the stem and two additional spots on the upper, and one on the lower fork; stem with a dark area, rather variable in extent on basal half. Sixth vein with a large dark area involving at least the outer half and a small spot usually present towards the base. Fringe with a small apical dark spot opposite about the lower branch of the second vein; pale spots present opposite the ends of the branches of the fourth and fifth vein; no spot at end of sixth vein. Wing scales relatively short and broad in comparison with those of the type form of *A. marshalli* and much as in *A. domicolus*.

Legs. Femora and tibiae dark-scaled except for very narrow pale rings at their apices and sometimes also at the bases of the latter. Front tarsi with narrow, apical, pale rings on first four segments; hind tarsi with rather broader pale rings at apices of first four segments and very narrow pale rings or indications of such at the bases of some or all of segments three to five.

Size. Wing length about 2.5 to 3.3 mm.

Pharyngeal armature (buccopharyngeal of Sinton, Barraud and Covell). This agrees with that of Group *Myzomyia* ('Class D' of Barraud and Covell, 1928), in having a double row of teeth, without deep-set roots. At least eleven pairs of teeth (rods and cones) can be

counted. The 'cones,' as seen from the dorsal surface, appear definitely flask-shaped, with the distal margin serrated into 3 or 4 pointed processes; the appearance could be readily distinguished from that seen in *A. moucheti* and *hargreavesi* which seem to resemble each other closely in the form of the 'cones.'

MALE. Palp with usual narrow pale ring at about middle and a rather large pale area on each segment of club and at its base.

Terminalia. No constant difference could be seen between the hypopygium of this species and that of the type form of *marshalli*.

FOURTH STAGE LARVA (fig. 6). Clypeal hairs all simple, outer anterior and posterior about half the length of the inner anterior. Mental plate with median tooth and laterally with three well-developed teeth and a very small proximal one.

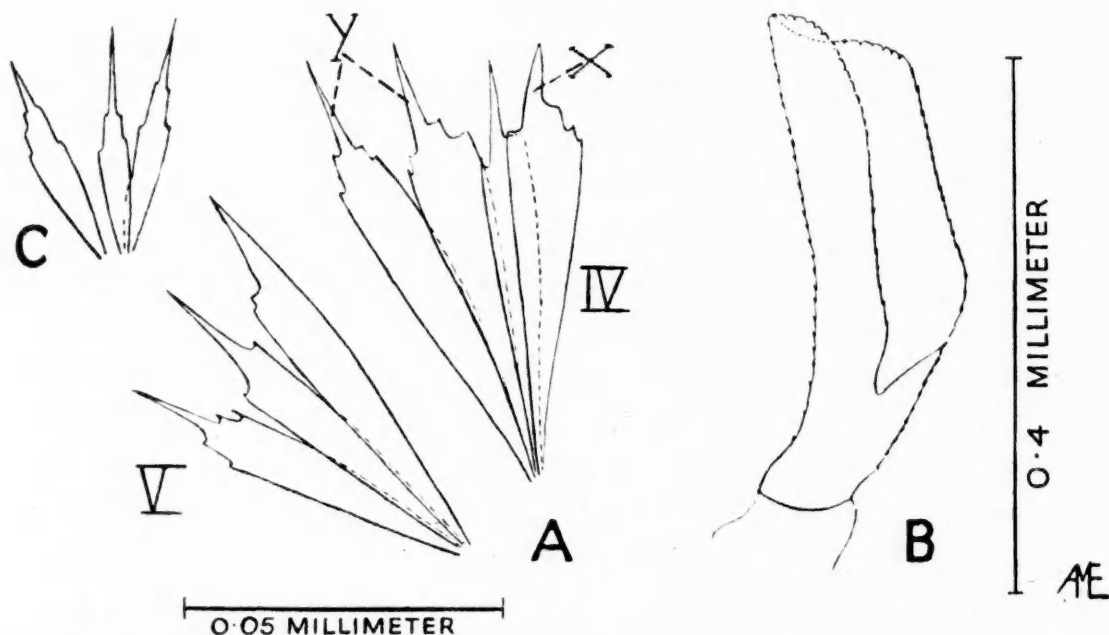


FIG. 6. *Anopheles barberellus*, n.sp. A.—leaflets of palmate bristles of fourth and fifth abdominal segments of full-grown larva; B.—respiratory trumpet of pupa; C.—leaflets of palmate bristles of first abdominal segment. A and C to the same scale.

Pleural hairs with the long bristles, as in *A. marshalli*, *moucheti* and *hargreavesi*, agreeing with those of Subdivision IV, Group 3 of Puri (1928). Prothoracic submedian bristles of normal character; inner and middle with their bases fused. Thoracic palmate bristle present; leaflets of the usual very narrow, shoulderless type. Abdominal tergal plates slightly larger than in *A. hargreavesi*. First to seventh abdominal segments with palmate bristles, those on first segment very small and with leaflets distinctly notched. Typical

palmate bristles with leaflets rather variable in shape, but usually much as in fig. 6, Y. Leaflets on fourth to sixth segments with ratio of length of filament to total length of leaflet usually about 1:3.8 to 1:5; exceptionally the filament is very short (X). Pecten with four long teeth alternating with groups of much shorter teeth, the ventral group being the most numerous in both specimens.

PUPA. As the following description is based on two specimens only, the smaller hairs are not dealt with. It is possible that some of the following characters may prove to be somewhat variable when further material becomes known.

Respiratory trumpet (fig. 6, B) narrow, with short meatus.

Abdominal chaetotaxy. Series A on fourth to seventh segments practically as in *A. moucheti*; normal on eighth. C, well-developed, simple and from one-half to practically the whole length of the succeeding segments on the fifth to seventh segments; moderately developed and 5 to 6; 4 to 7, and 3- to 5-branched on the second to fourth segments respectively. B, about half as long as succeeding segment and simple to trifid on sixth and seventh; bifid to quadruple on fifth; moderately developed and 5- and 5- or 4-branched on third and fourth segments. C', on sixth segment very slender and apparently simple.

Paddles not very well displayed in either specimen; fringe apparently only present externally to the apical bristle; spine-like processes scarcely apparent on proximal third, but a few of these present at about the middle of the external border and merging rather gradually into the long fine distal hairs. Apical bristle bent into a hook (incomplete in one pelt). Subapical hair bifid distally.

Cotypes: one ♀, together with pelts of pupa and larva: hillside spring, Firestone Plantations, Liberia, 15.iii.31, Dr. M. A. Barber; one ♀, together with pelt of pupa and an associated larva taken in ditch, Charlotte Road, Regent, above Freetown, Sierra Leone, 8.ix.25, A. M. Evans (see Blacklock and Evans, 1926, and Evans, 1929, p. 423); one ♂ among a collection reared from larvae taken in Nicol's Brook, Whitewater Bridge, Freetown, Nov. 1927-Jan. 1928, by Professor R. M. Gordon. A specimen taken at Iketobo, S. Nigeria, 1913, by Dr. J. W. S. Macfie has been identified as this species by Dr. F. W. Edwards. Other examples from Sierra Leone probably referable to *A. barberellus* are:—one ♀ bred from larva taken in

a swamp at Mabang, 1926, and one ♂ bred out at Kissy, near Freetown, 25.xii.30, by Professor R. M. Gordon; one ♀ reared from a larva taken at Wilberforce, Freetown, 1925, by the writer.

This species is named in honour of Dr. M. A. Barber, who has bred it from the larva, apparently for the first time.

A. barberellus resembles *A. domicolus* Edw. most closely among the species of the *marshalli* group, and specimens of it were provisionally referred to this form. Dr. Edwards, who kindly examined my material and compared it with the type series of *domicolus*, concluded that the present form is a distinct species, differing from *domicolus* mainly as follows:—Hind tarsal rings much narrower than in *domicolus* and basal banding much less definitely developed; wing with basal portion of costal region much paler than in *domicolus* (two well-marked pale interruptions present in *barberellus*).

ANOPHELES OBSCURUS var. *NOWLINI* n.var.

This variety differs from the type form of *obscurus* as follows:—

FEMALE AND MALE. All tarsal segments entirely dark in all specimens. Wings constantly with three or four small pale spots on about the basal quarter of first vein.

Male terminalia. The one ♂ received was a very small specimen and the hypopygium was also of small size. It differed markedly from other *obscurus* material examined, in all of which the leaflets were relatively very long (more than half the length of the phallosome), a character first pointed out by Christophers (1924). In the specimen of var. *nowlini* the leaflets are relatively much shorter, the longest being little more than one-third the length of the phallosome. There are about four pairs of leaflets, all very narrow, the two smaller ones bristle-like.

FOURTH STAGE LARVA. The chief differences from the type form of *obscurus* from S. Nigeria (see Evans, 1931 *a*) are:—(1) the character of the apical antennal hair which in this variety is of normal length and form, being considerably longer than the two spine-like processes at the apex and having not more than eleven branches, all of which are simple. In West African *obscurus* this hair is not longer than the spines and its branches are secondarily divided, resulting in about 18 to 25 ultimate branches; in East

African material, received from Mr. G. H. E. Hopkins, the hair is very short and shows some tendency to secondary branching though not so markedly as in Dr. Barber's Lagos and Liberian material. (2) The outer clypeal hairs are very variable in length and branching; in some specimens they are about equal to those of typical *obscurus* in size and shape but the number of branches does not exceed about 7 (10 to 20 in typical *obscurus* from West Africa and up to 30 in East African material). In the majority of specimens, however, the outer clypeals are very short, and frequently only about one-quarter the length of the inner pair and simple or bifid. Another difference from W. African *obscurus* type form concerns the shape of the abdominal tergal plates, which tend to be relatively shallower than in the type.

PUPA. This differs from that of the type form in having the smaller dorsal lobe of the trumpet (compare with fig. 2, B) reduced to a narrow tongue with breadth only about one-third length.

Cotypes: 3 ♀♀, 1 ♂ with larval and, in two cases, pupal pelts, the larvae collected in clear wooded hill streams. Other material:—2 ♀♀ bred from larvae; two collections of larvae from similar wooded streams, associated with larvae of the type form of *obscurus* and, in one instance, with those of *A. cinctus*. All material collected on the Firestone Plantations, Liberia, February and March, 1931, by Dr. M. A. Barber.

Larval habits. Dr. Barber noted that larvae of this form showed a great tendency to cling to stones and rocks in the stream, reminding him in this respect of those of *Chagasia fajardoi*.

This variety is named in honour of Mrs. M. A. Barber (née Nowlin), who rendered considerable assistance to Dr. Barber during his entomological investigations in Nigeria.

Some of the characters of this variety seem, perhaps, to indicate specific distinction from the type form, but as this tends to be variable in different parts of its range, it seems better to regard the present form as a variety until more larval material of the type has been studied.

Reference has been made above and elsewhere to small differences in larval and pupal characters between Dr. Barber's material of typical *obscurus* from West Africa (Lagos and Liberia), and specimens sent by Mr. G. H. E. Hopkins from Uganda. As the type of

strahani Theo. is from Lagos and that of *obscurus* Grünb. from the Cameroons, it seems probable that Dr. Barber's specimens represent the type form in its strictest sense.

A distinct difference has also been observed between the phallosome leaflets of this form, on the one hand, and of ♂♂ from Uganda (Mr. Hopkins' specimens) and the Belgian Congo on the other. In Congo and Uganda material studied, the phallosome bears at each side about two or three narrow, blade-like leaflets and a number of narrow bristle-like appendages, some of these about as long as the true leaflets and others much shorter. In West African material examined, however, long bristles are absent, the phallosome bearing groups of two to four leaflets, the longer ones blade-like, though narrow; the very short leaflets may be so narrow as to be practically bristle-like. As, however, these differences and those seen in the larvae and pupae of East and West African forms are fine, it seems best to wait until much more material has been studied before regarding the Central and East African form as a distinct race or variety of *obscurus*.

ANOPHELES CINCTUS Newstead and Carter

Since the discovery in 1910 or earlier of the type of this species, no other specimens appear to have been collected until Dr. Barber reared specimens of it from larvae which he found in streams in Liberia early in 1931. The adults bred out included four males and three females, mostly in a good state of preservation and these, together with a good series of larvae and larval and pupal pelts, have been very kindly sent to us by Dr. Barber. It is, therefore, possible to add a considerable amount to the original description which was made from a single incomplete female. Examination of this series shows that the markings of the last hind tarsal segment show some variation so that the characters given in keys to the adult anophelines will have to be revised. It is interesting to note that Newstead and Carter's supposition that the female palpi would show four pale bands is found to be correct. On the other hand, the characters of the female pharyngeal armature and of the larval pleural bristles show that the species should be placed in the group *Neomyzomyia*, not *Cellia* as formerly supposed.

The following description is supplementary to that given by Newstead and Carter:

FEMALE. Palps mainly clothed with dark, blackish scales, very shaggy on first three segments; each of last four segments with a narrow, pale, apical band. The five segments respectively about: 5, 27, 36, 20 and 12 per cent. of the whole organ. The flagellar segments of the antenna may be practically black.

Thorax. Anterior half to two-thirds of mesonotum densely clothed with broad, flat, mainly truncated scales, whitish or greyish yellow in colour and rather transparent; fossae with several broad, truncated scales; the broad scales of the mesonotum abruptly replaced behind by quite narrow, curved scales. One proepimeral bristle present.

Wings. On the whole the wing markings of the specimens agreed to a very large extent with the account of those of the type. The chief differences or variations observed were as follows:— In all specimens the costa showed the five dark areas spreading on to the first vein, but the pale interruption on the costa and first vein at the end of the subcosta was considerably shorter than in the illustration given by Newstead and Carter, being not more than half the dark area beyond it; this was also noted in the wing of the type. The first vein showed one or two additional dark areas in the basal region. The second vein was usually as described for the type but on one or two wings a small pale interruption was present near the middle of the upper fork; in one specimen one wing had this vein mainly pale. In the lower branch some of the scales of the pale area may be grey, making the actual extent of the light and dark areas rather doubtful. The third vein showed one or two dark spots basally, in addition to the apical spot; in one specimen the sub-basal spot was prolonged forward by a light grey scaled area. The fourth and fifth veins usually have the same number of dark spots as described for the type but these are liable to some variation in extent and there may be a rather extensive dark area towards the base of the fourth vein. The sixth vein may have the outer half entirely dark or with a pale interruption. There is usually an additional pale fringe spot about half way between the apex of the sixth vein and the base of the wing. A dark spot is usually present at the apex of the wing near the end of the lower branch of the second vein.

Legs. Front, mid and hind legs with femora and tibiae

conspicuously spotted and ringed with yellow or yellowish-white scales. Front tarsi with about 4 to 6 very distinct pale rings on first segment ; second to fourth segments usually with a dark ring at the base ; second sometimes with an additional dark ring ; fifth segment entirely pale in all the specimens. Mid-tarsi practically as those of front legs. Hind tarsi with about seven or eight distinct pale rings on first segment, three or four on second, two or three on third ; fourth variable, a dark ring always present at base, rest of segment may be entirely pale or show one or two additional dark rings. Last segment also variable, either entirely pale or with a more or less complete dark ring at the base.

Abdomen. Integument usually blackish brown, sometimes paler towards posterior margins. Scaling much as described for the type. Scales confined to distal half or less of segments two to six ; blackish brown scales forming outstanding tufts and extending dorsally, replaced by creamy or greyish white scales which occupy about the middle third. Seventh segment with scaled area more extensive ; amount of light and dark scaling somewhat variable. Eighth segment covered with pale scales which may be partly ochraceous and partly creamy white. Venter of eighth segment with pale scales.

Pharyngeal armature (one specimen examined). The armature consists of a row of about 8 large, pectinate teeth with well separated, stout bases ; teeth not differentiated into 'rods and cones.' The teeth project upwards at a considerable angle from the plane of the pharynx so that it is not possible to give a very detailed description of them, but their terminal processes seem to be rounded. Two pairs of papillae are situated in front of the teeth, and the dorsal papillae appear to consist of two pairs and an unpaired one but it is possible that one has been broken off.

The character of the armature clearly indicates affinities with the *Neomyzomyia* group. Professor Patton kindly examined the structure and arrived independently at this conclusion.

MALE. Palps. Club with a moderately large, pale area on each segment and a pale spot at the base ; no pale scales can be seen in the position of the usual ring at the middle of the stem.

Terminalia (two specimens examined). Parabasal spines 4 or 5 in number and none of them markedly stouter than the rest. At least 3 may be bent in their distal portions but the bent appearance

is much less marked than in such a typical *Myzomyia* as *A. funestus*; the shorter spines are relatively longer than in *funestus*. Basal lobe of coxite (harpago, Christophers and Puri, 1931) with club very slender and slightly dilated; apical hair about as long as club; no well developed accessory hairs seen in either preparation. Phallosome relatively short and broad and *without* leaflets. Tenth or anal segment with microtrichia of membrane very conspicuous.

FOURTH STAGE LARVA (fig. 7). A very small, rather dark larva, length about 3 to 3.5 mm.; colouration (in formalin preserved specimens): ground colour pale olivaceous with much pigment of dark olive colour dorsally; head capsule deep mahogany brown to sepia.

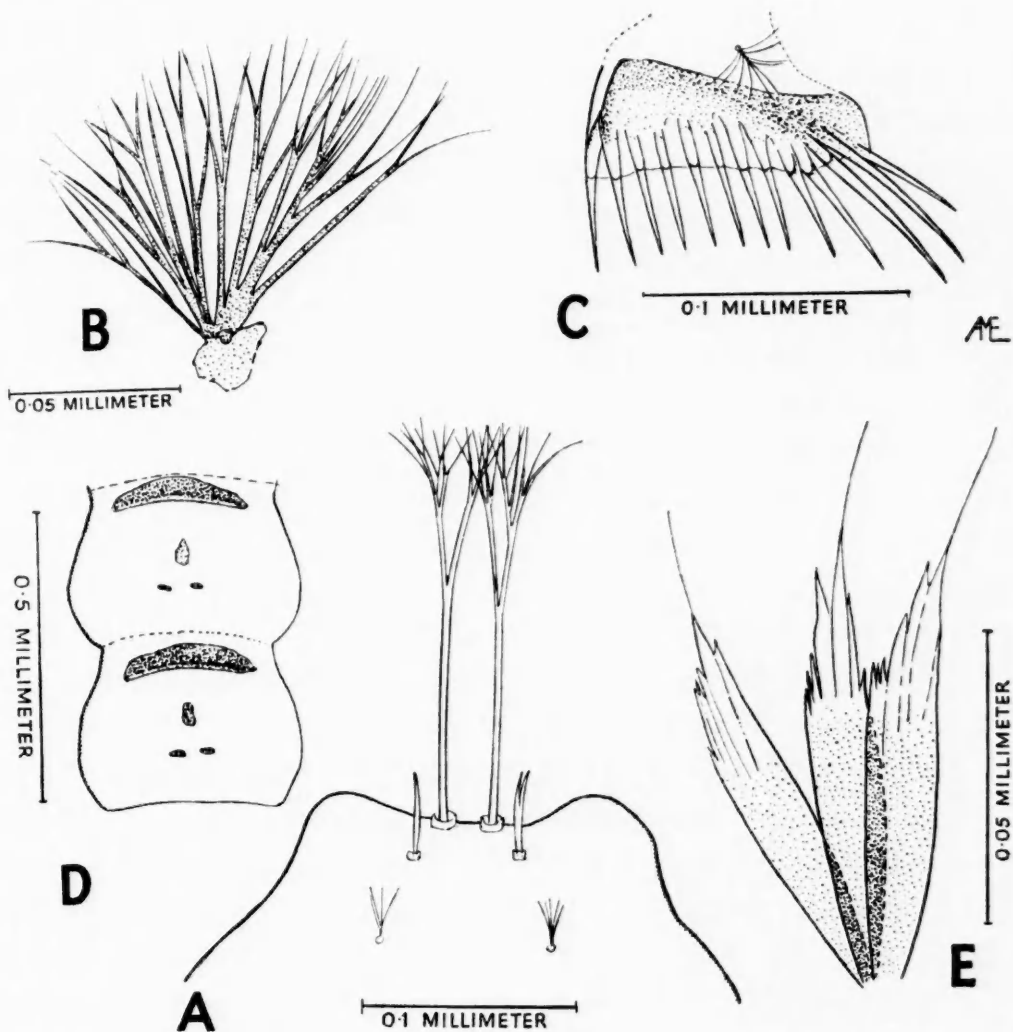


FIG. 7. *Anopheles cinctus* Newstead and Carter. Details of fourth-stage larva. A.—clypeal hairs; B.—inner submedian prothoracic bristle of left side; C.—pecten; D.—outline of sixth and seventh abdominal segments showing tergal plates; E.—leaflets of palmate bristle of fourth abdominal segment.

Head slightly broader than long; much widened and rounded at level of eyes. Fronto-clypeus usually with dark ground colour, sometimes with more or less distinct darker markings, the best defined being a rounded median spot and two oval lateral areas on the posterior half, which tend to merge into the dark border of the front. *Inner clypeal* bristles situated rather near together (fig. 7, A), long and branched at about the distal half or rather before this; the first branching usually results in 2 or 3 main branches which redivide, giving a total of about 8 to 12 ultimate branches. Outer clypeal hairs placed in a very unusual position, close to inner but slightly behind the bases of these; very short and somewhat thickened; usually either simple or bifid. Posterior clypeals (anterior frontals) placed considerably behind the anterior; and *outside* the outer anterior they are usually branched (about 2 to 5). *Antenna* with prominent, often darkly pigmented spicules on ventral surface and along inner border; hair on shaft very minute, simple; apical hair extremely delicate and with about 7 to 14 (usually 9 or 10) branches, which are not well seen in specimens mounted dorso-ventrally. The two inner mid frontal bristles with branches long and tending to rise from nearer the base than usual. Posterior frontal usually simple or bifid; vertical usually bifid, sometimes 3- or 4-branched. Mental plate with a central tooth and five lateral ones at each side, that next the median shorter than the succeeding tooth. Plate ventral to mental plate, i.e., submentum (Salem, 1931, and Puri, 1931) relatively thin and unchitinised; teeth poorly developed.

Inner submedian prothoracic bristles (fig. 7, B) dendritic with short, flattened stem from which arise numerous fine branches, all about the same length and most of them bifurcated or branched; inner and middle arising from separated bases. Bristle external to submedian group with branches stout and relatively few. Thoracic palmate bristles relatively large with numerous narrow, unshouldered leaflets. *Pleural bristles*, Prothoracic: anterior dorsal long and feathered but not specially stout; anterior and posterior ventral long, slender, simple; posterior dorsal one-third ventral, and with about 4 branches arising near the base. Mesothoracic: anterior dorsal and ventral long, slender and simple. Metathoracic: anterior dorsal and ventral long, slender and simple, but distinctly shorter

than usual, being only about four-fifths as long as the feathered lateral bristles of this segment.

Abdomen with lateral plumose hairs long and well developed on the first three segments. Palmate bristles small on first segment, but with leaflets similar to those of others; on segments two to seven, large and well developed. Typical palmate bristles (fig. 7, E) with main portion of leaflet pigmented, usually darker near the base but with outer jagged portion extremely transparent and longitudinally striated; some of jags produced into very fine points, and terminal filament extremely fine, resembling cilia. Owing to the extreme delicacy and transparency of the outer portion of the leaflets, these appear in larval pelts to be produced distally into a fringe; this effect is caused by the striae, the membrane of the outer part being invisible in pelts. Abdominal tergal plates (fig. 7, D) very broad in a lateral direction and shallow antero-posteriorly. Second to seventh segments with three small posterior plates arranged as in fig. 7, D. Pecten with 12 to 14 teeth of which the three or four dorsal ones are elongated, the rest being of practically uniform length (fig. 7, C). Saddle hair (lateral on tenth tergite) branched (3 to 5). Inner and outer clinging bristles (Patton and Evans, 1928) both with longer branches curved to form hooks; inner relatively short, only slightly more than half the outer. Outer with only two long stout branches arising dorsally.

PUPA. Respiratory trumpet relatively narrow; meatus slightly more than one-fifth the total length of trumpet.

Abdominal chaetotaxy. Bristle *A*, on eighth segment long and with numerous fine bilateral branches; on seventh very slender, finely pointed, and about half as long as the next segment; on fifth and sixth also slender and finely pointed. *B* and *C* are moderately short, not exceeding about three-quarters of the succeeding segment and usually less than this. *B*, on segments four to seven consists of a short stem with closely set, bilateral branches, usually too dense to be counted and, in three of the four specimens, rather coarse and darkly pigmented, the whole bristle appearing somewhat as in fig. 2, D; on segment three the stem is longer with about 15 to 21 long delicate branches, some of which may be bifurcated. *C*, similar to *B*, on sixth and seventh; tufts moderately pale and with about 6 to 9 branches on fourth and fifth;

on second and third with 8 to 13 delicate branches, some bifid. Other hairs relatively small, including those along anterior border of first segment.

Paddles. Apparently rather narrow and with distal margins more rounded than usual, but this appearance may be due, to a slight extent, to the orientation of the mounts. Fringe of delicate hairs extending along most of margin, from the base internally round the distal border and for more than two-thirds of the external border, the basal portion of which is without any processes; hairs relatively long on distal border, some being at least half the length of the apical bristle. Apical bristle bent or curved distally but not markedly hooked as in *funestus*, etc.; subapical hair split into two or more delicate branches.

Description based on 4 ♂♂ and 3 ♀♀ bred out from larvae, 3 larval and 4 pupal pelts and several larvae. Larvae collected in wooded hill streams on the Firestone Plantation, Liberia, February, 1931, by Dr. M. A. Barber.

The characters of the pharyngeal armature and larval pleural bristles clearly show that this species should be placed in the *Neomyzomyia* group of the subgenus *Myzomyia*. The occurrence of abdominal scale-tufts is, therefore, again shown to be no certain indication of affinity among the species of this subgenus, possessing this character (group *Cellia*, see also Barraud and Covell, 1928). The larva shows very unusual features, some of which can be more or less paralleled among other species of the *Neomyzomyia* group. The approximation of the inner clypeal hairs is met with also in *nili*; the rounded, dendroid condition of the inner submedian prothoracic bristle is approached by that of *natalensis* and *multicinctus* (specimens received from Mr. Hopkins), in which the central stem is very short and broad; the relatively broad though shallow tergal plates are also a feature in common with these species and with *nili*. These peculiarities in *natalensis* were first pointed out by Mr. De Meillon. Other peculiar features of the larva of *cinctus* are the position of the outer clypeals whose bases are within those of the posterior pair; the extreme fineness of the filaments of the palmate bristles.

III. THE POSITION OF *ANOPHELES FREETOWNENSIS* Evans

Anopheles marshalli var. *freetownensis*, Evans (1925), which was raised to specific rank by Christophers and Puri (1931), can no longer be regarded as even belonging to the *marshalli* series of *Anopheles*, nor is it a typical member of the *Myzomyia* group as defined on the character of the pharyngeal armature (Barraud and Covell, 1928).

Wing markings. A re-examination of material of the species collected in Sierra Leone, shows that the basal region of the costa normally has two pale interruptions, one or both of which are well marked.

Pharyngeal armature. About 14 pairs of teeth present showing definite 'rod and cone' arrangement. Bases of cones narrowed to form slender 'roots' which, however, project from the chitinated wall of the pharynx and do not appear to be embedded in it as in *pharoensis* and *costalis*.

Larval pleural bristles. The character of the pleural bristles places this species in the same group as *sergenti*, *rhodesiensis*, *transvaalensis*, etc., the only difference from *sergenti* being that the feathered anterior dorsal bristle of the metathoracic group is slender rather than stout.

The characters of the pharynx and pleural bristles of a number of other African species are being investigated and it is hoped to publish some interesting results in the course of time.

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EXPERIMENTS ON THE TRANSMISSION OF *TRYPANOSOMA BRUCEI* AND *TRYPANOSOMA RHODESIENSE* TO MAN

BY

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INTRODUCTION

One of the chief problems in the study of human trypanosomiasis is the relationship between the three polymorphic trypanosomes, *T. gambiense*, *T. rhodesiense* and *T. brucei*. They are indistinguishable from one another morphologically. The two former infect man but are distinguished roughly by the fact that, in subinoculated laboratory animals, *T. rhodesiense* nearly always produces numerous posterior-nuclear forms while *T. gambiense* produces none or very few. Furthermore, *T. rhodesiense* is more susceptible to the action of human blood serum, as tested in rats or mice or *in vitro*, than *T. gambiense*. In the production of numerous posterior-nuclear forms and in its susceptibility to human blood serum, *T. brucei* resembles *T. rhodesiense*, and the two trypanosomes are only distinguished by the fact that one infects man and the other is not known to be able to do so. Attempts to distinguish *T. rhodesiense* from *T. brucei* by various serological tests, have not hitherto met with much success. From the point of view of practical tropical hygiene, three important questions need investigation. One is whether *T. gambiense* becomes changed in its distinguishing characters by repeated cyclical passages through such tsetse flies as *Glossina morsitans*, *G. pallidipes* and *G. swynnertoni*. The second is whether

T. brucei, occurring in areas where human trypanosomiasis is unknown, can infect man. The third question is what changes may occur in *T. rhodesiense* by a prolonged stay in the bodies of lower animals. The two latter questions may be summarised by saying that we need to find out if *T. brucei* can become *T. rhodesiense* or *T. gambiense*, and if *T. rhodesiense* can become *T. brucei*. A stage has now been reached where experiments on man are required. Taute's well-known experiments showed that a few strains of *T. brucei* failed to infect any one of a considerable number of men. Those strains can only be called *T. brucei*, as there is at present no justification for giving the name *T. rhodesiense* to any strain of trypanosome that has not infected man. Considered together with the high percentage of antelopes infected with polymorphic trypanosomes and with the distribution of human trypanosomiasis, Taute's experiments support the working hypothesis that there is a polymorphic trypanosome of animals, *T. brucei*, that cannot infect man, and another, *T. rhodesiense*, that infects man and lower animals also. One of the most promising lines of research would be to transmit *T. rhodesiense* by one of the species of game tsetse flies through a series of antelopes, and finally to see if it had lost its infectivity for man; in other words, if it had become changed into *T. brucei*. Such an experiment presents no real difficulties. The following experiments with *T. brucei* and *T. rhodesiense* were carried out at Tinde.

EXPERIMENTS WITH *T. BRUCEI*

The strain used. I am indebted to Mr. H. E. Hornby, O.B.E., F.R.C.V.S., Acting Director of the Veterinary Department of Tanganyika Territory, for the strain of *T. brucei* and for details of its history. The strain was isolated in April, 1927, from a cow which had travelled through areas of Kahama district infested with tsetse flies. The cow was almost unaffected by the disease. The subsequent transmissions by inoculation during four and a half years may be summarised thus:—cow, rat, ten guinea-pigs, rat, guinea-pig, eight rats, guinea-pig, rat, guinea-pig, rat, two guinea-pigs, two rats, rabbit, five guinea-pigs, rat, nine goats and five guinea-pigs. Mr. Hornby has suggested in his Annual Report

that this trypanosome may be *T. rhodesiense*, from the fact that the infection of the cow apparently occurred in the Kahama district early in 1927. Sleeping sickness in Kahama district, however, was not found until February, 1928, so that the chances of this strain having previously infected man are very slight.

Transmission experiments. Laboratory-bred tsetse flies, consisting of a mixture of *G. morsitans* and *G. pallidipes*, were fed for a week on guinea-pigs infected with the strain of *T. brucei*. They were then re-arranged in batches in bottles and fed on four clean guinea-pigs, all of which became infected. The remaining flies, forty-eight in number, were then fed on my arm for three days. Fifty-three feedings were counted, and, so far as could be observed, all the flies fed. In the case of forty of the flies the interval of time since the last opportunity of feeding on the infected guinea-pigs was from the twenty-fourth to the twenty-sixth day, and in the case of the other eight flies, from the twenty-eighth to the thirtieth day. There was therefore time for the salivary glands to have become infected in all infected flies. The flies were then put to feed on eight fresh guinea-pigs, no fly, of course, being allowed to feed on more than one guinea-pig. Three of the eight guinea-pigs became infected. There must therefore have been at least three infective flies. The remaining flies of the bottles corresponding to the three infected guinea-pigs, reduced in number by deaths, were then placed singly in bottles and put to feed on a second European volunteer, Mr. H. C. Smith, of the Veterinary Department. Afterwards they were put to feed on fresh guinea-pigs and then were killed and the salivary glands examined by the method of Lloyd and Johnson. Only one fly showed infected salivary glands, and the corresponding guinea-pig, fed on six days after the fly had fed on Mr. Smith, became infected. In this case the fly fed on Mr. Smith on the forty-ninth day after its last opportunity of feeding on an infected guinea-pig. Neither of the volunteers became infected.

Comment. It is very probable that at least three infective flies fed on the first volunteer, and it is practically certain that one infective fly fed on the second volunteer. It is of some interest that a strain of *T. brucei*, that had been maintained by inoculation in animals for four and a half years, was readily transmissible by tsetse flies.

THE TRANSMISSION OF *T. RHODESIENSE* TO MAN

The strain used. The strain of *T. rhodesiense* used, Strain 271, Bupamba, was inoculated directly from man into goats on March 25th, 1930, at Maswa, Tanganyika Territory. It had since been maintained by direct inoculation, first in a series of goats and later in a few sheep. The chain of transmission in goats was broken by two passages in rats in February, 1931, on the transfer of work from Maswa to Tinde. The series of transmissions by inoculation may be summarised thus:—Native Bupamba, fourteen goats, two rats, goat, three sheep. At the time of the experiment the trypanosome had been maintained in these animals for nineteen months. This strain has been the subject of previous observations. After maintenance in goats for three and six months it showed a greater susceptibility to human serum, as tested in subinoculated rats, than fresh strains of *T. rhodesiense* from man. The susceptibility to human serum was again tested sixteen months after its isolation from man, and was found to be much the same as at six months. I am permitted by Dr. A. R. D. Adams to record that he tested this strain of trypanosome against normal human serum at Entebbe, Uganda, in September, 1931, using the *in vitro* technique of Yorke, Adams and Murgatroyd. He found that it was not resistant, all trypanosomes, so far as could be judged by microscopical examination, being killed within six hours by undiluted normal human serum.

The transmission experiment. On October 23rd, 1931, a 'Bruce fly box' containing about twenty laboratory-bred tsetse flies, consisting of a mixture of *G. morsitans* and *G. pallidipes*, was applied daily to sheep T.7, infected with *T. rhodesiense*, Strain 271, Bupamba. On October 25th, sheep T.8, infected from sheep T.7, was substituted for sheep T.7, as the latter was dying. More tsetse flies were added during the next few days. On November 6th, a clean goat was substituted for sheep T.8. This goat did not become infected. On November 14th, there were thirty-six remaining flies, and these were divided into batches of six in six bottles and fed on six guinea-pigs, Nos. 11, 12, 13, 14, 15 and 16. Three of the guinea-pigs, viz. :—Nos. 11, 13 and 14, became infected. The remaining flies of the bottles corresponding to the three infected guinea-pigs were put

to feed on my arm and afterwards on clean guinea-pigs. The flies were afterwards dissected so far as was possible. The only evidence thus obtained was in one of the flies that had infected guinea-pig No. 13. This fly had fed on my arm on December 1st, but refused to feed subsequently on a clean guinea-pig. It was dissected on December 11th, and its salivary glands were found to be infected. The salivary glands, in salt solution, were injected into a rat on December 11th and infected it. This fly had last had an opportunity of feeding on the infected sheep on November 6th, twenty-five days before it fed on man and thirty-five days before its salivary glands were found to be infected. It is therefore not certain that the salivary glands were infective on the day, December 1st, on which the fly fed on man.

I decided to inoculate myself from guinea-pig No. 13. The susceptibility of the strain of trypanosomes to human serum after its cyclical passage through the tsetse fly was tested. On November 30th, three rats were inoculated directly from a sheep infected with this strain of trypanosome, and three other rats were inoculated from guinea-pigs which had been infected with the strain by means of tsetse flies. One of the rats, inoculated from a guinea-pig, showed a long incubation period, over twelve days, and trypanosomes were so scanty in its blood on the day of the test that it was not used. The other five rats were injected with human blood serum, including my own, and the results are shown in the following table.

TABLE

Rat	Inoculated on	Inoculated from	Serum injected on	Serum of	Amount c.cm.	Number of trypanosomes per field, thin stained film, No. 7 Ocular, 1/12 Objective						
						Before injection of serum	After injection—days					
							1	2	3	4	5	6
78	30.11.31	Guinea-pig D.	19.12.31	Native M. ...	2	35	30	2	1/3	1	10	45
78b	30.11.31	Guinea-pig 14 ...	19.12.31	J.F.C. ...	2	8	1/20	1/200	1/4	1	3	7
79	30.11.31	Sheep ...	19.12.31	Native M. ...	2	20	6	2/3	1/20	1/4	1/2	1
79a	30.11.31	Sheep ...	19.12.31	J.F.C. ...	2	8	1/3	0/400	0/400	1/200	1/6	2
79b	30.11.31	Sheep ...	22.12.31	Native P.B. ...	2	7	1/100	0/400	0/400	1/400	3/400	1/10

All that can be concluded from the test is that the strain of trypanosome showed some susceptibility to the action of human blood serum. It appears as if passage through tsetse fly into a guinea-pig had resulted in a somewhat lessened susceptibility, but the experimental evidence is too small to base conclusions on. The blood of rats 478, 479, 479a and 479b showed numerous posterior-nuclear forms.

On December 19th, a few drops of blood from an ear vein of guinea-pig No. 13 were taken into a little citrated saline. A drop of the mixture was examined under the microscope and living trypanosomes were seen. Then I injected a few drops of the mixture subcutaneously into my arm and also into a control rat. On the next day there was an erythematous area, about one and a half inches in diameter, over the point of injection into the arm. On the two following days this was absent, but reappeared on December 23rd without pain. On December 24th, this area was larger and of a deeper red colour, and I had a feeling of malaise. On December 25th, the area had increased further in size and showed a slight appearance of tension, suggesting that an abscess might develop. A stained thick blood film showed no trypanosomes. On the evening of December 25th, I had a slight rigor and felt unwell. It was not convenient to take a blood film. On December 26th, a stained thick blood film showed two trypanosomes, but none could be seen in a fresh preparation. Aspiration of the erythematous area of the arm, by the intradermal insertion of a hypodermic needle attached to a syringe, performed by Dr. G. Maclean, Sleeping Sickness Officer, gave a smear that showed several trypanosomes after staining. My temperature was now 101.8°F . In the evening of December 26th, eleven rats were inoculated with blood taken from an arm vein. Treatment was postponed as living trypanosomes had not been found in my blood. On December 27th, my temperature was 103°F ., and Dr. Maclean found a living trypanosome in a fresh blood preparation and several well-stained trypanosomes in thick blood films. He inoculated three more rats, and then began treatment by intravenous injection of Bayer 205. The erythematous area on the arm gradually disappeared without any complications. Ten of the eleven rats, inoculated on December 26th, became infected, as did all three rats inoculated on December 27th. The blood of these

rats showed numerous posterior-nuclear forms. The control rat, inoculated on December 19th, became infected on December 24th, and showed numerous posterior-nuclear forms in its blood.

SUMMARY AND CONCLUSIONS

1. A strain of *T. brucei* that had been maintained by direct inoculation in various animals for four and a half years was found to be easily transmissible by tsetse flies to guinea-pigs. Two European volunteers did not become infected after having been bitten by these infective flies.

2. A strain of *T. rhodesiense*, that had been maintained by direct inoculation in goats and sheep for nineteen months, was found to be easily transmissible by tsetse flies to guinea-pigs. A European volunteer, inoculated subcutaneously with the blood of one of the infected guinea-pigs, became infected, the incubation period being six or seven days. The infection was transmitted from the infected man to rats by inoculation of blood. It may be said that a strain of *T. rhodesiense* had not become converted into *T. brucei* by a stay of nineteen months in the bodies of goats and sheep.

3. It would seem that domestic animals need to be taken into account as possible means of spread of sleeping sickness.

4. It is very desirable that work with antelopes should be undertaken in order to advance our knowledge of the subject of animal reservoirs of human trypanosomes.

MISCELLANEA

THE MOLLUSCAN HOST OF *S. HAEMATOBIIUM* IN NORTHERN NIGERIA

The molluscan host of *S. haematobium* in Nigeria has not yet, so far as the writer is aware, been described. This omission is of some importance as the disease is extremely common amongst the native population in various parts of the Colony. Blacklock (1924) has shown that in Sierra Leone the snail responsible is *Physopsis globosa*.



In view of Blacklock's discovery it appears of interest to record that, while spending a few days at Kano in Northern Nigeria, the writer was able to demonstrate the presence of this species of snail in the numerous small ponds which occur within the circumference of the walls which surround this large native city. The accompanying photograph illustrates a typical pond of this nature and shows how the native children who bathe and fish in its waters are exposed to infection. On making inquiries it was found that a very high proportion of the children in Kano were suffering from the disease.

The writer is greatly indebted to the Government of Nigeria, for granting travelling facilities to Mr. Warminger of the Sanitary Department for collecting various species of snails, and to Major M. Connolly of the British Museum for confirming the identification of the *Physopsis globosa* captured.

R. M. GORDON.

REFERENCE

- BLACKLOCK, D. B. (1924). Report on an investigation into the prevalence and transmission of human schistosomiasis in Sierra Leone.

STUDIES ON THE TRANSMISSION OF EXPERIMENTAL YELLOW FEVER BY *CULEX THALASSIUS* AND *MANSONIA* *UNIFORMIS**

BY

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Following the original demonstration by Bauer (1928), that mosquitoes other than *Aedes aegypti* are capable of transmitting yellow fever, a total of seven West African species were shown experimentally to be vectors of this disease. Transmission experiments with two additional species—*Culex* (*Culex*) *thalassius* Theobald and *Mansonia* (*Mansonioides*) *uniformis* Theobald—are here reported. Investigation of these species was deemed important because they are abundant, at least in parts of West Africa, because they enter houses readily, and because they bite man with great frequency.

TECHNIQUE OF TRANSMISSION EXPERIMENTS AND EXPLANATION OF PROTOCOLS

The experiments were carried out in the same general way as those of Bauer (1928) and Philip (1929, 1930a, 1930b). The female mosquitoes which were used in the tests were individually identified by the author with the aid of an $\times 10$ hand lens. After being exposed to an infectious monkey, they were examined by transmitted light to make certain that they had engorged blood. After the lapse of a number of days, considered sufficient to cover the necessary extrinsic incubation period, infectivity tests were performed. These were of two types. In the first and more important type of test, infected (?) females were allowed to engorge upon normal monkeys, in order to determine whether or not they could transmit the virus

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

of yellow fever by their bites. In the second type, infected (?) females were emulsified and the emulsion inoculated subcutaneously into monkeys. This was frequently done with female mosquitoes immediately after a test biting in order to determine whether they actually contained virus. Furthermore, only this type of test was possible with certain lots, which could not be induced to engorge a second time. The mosquitoes that were to be inoculated were killed either with tobacco smoke or by freezing. The freshly killed females were emulsified in sterile blood serum from normal rhesus monkeys, either undiluted, or diluted to not less than 10 per cent. with distilled water or physiological sodium chloride solution.

All the monkeys used in these experiments were *Macacus rhesus*. The infectious monkeys from which the mosquitoes secured their meal of infectious blood all developed their initial fever in from two to four days after they had been inoculated with virus, and all died of typical yellow fever in a few days, except the one upon which Lot 393 engorged. This monkey had a very severe febrile reaction, but recovered. It proved, however, to be a good source of virus. The mosquitoes were fed at the onset of fever in the infectious monkeys. A temperature of 40° C. or over was considered to be fever. After the infectivity tests the monkeys were observed for at least three weeks before they were given immunity tests, which consisted of the subcutaneous inoculation of a large amount of yellow fever virus.

The Asibi strain of virus, which is very virulent for *Macacus rhesus*, was used throughout these experiments.

CULEX THALASSIUS

Notes on Life Habits. *Culex thalassius* occurs throughout tropical and South Africa (Edwards, 1916-17), but it appears to be confined to a relatively narrow zone along the coast. Ingram and Macfie (1917-18) and Dalziel (1920-21) found that it breeds in small numbers in a great variety of places, and in large numbers in the shallow brackish, or salt, water at the edges of some of the extensive lagoons which occur along the West African coast. It has not been abundant in Lagos in recent years (Beeuwkes, Kerr and Weathersbee, and Dalziel, 1920-21). At various times at Takoradi and at Accra, Gold Coast, it has been reported to be very common both inside and outside

houses, and to attack man frequently (Macfie and Ingram, 1916-17, and a personal communication). The author has found that this species is active throughout the night, but that its greatest activity does not begin until about two hours after sunset.

Transmission Experiments. The first indication that *C. thalassius* might be a vector of yellow fever was the result of an experiment performed by Philip in 1929 (1930). Two females that had engorged upon an infectious monkey twelve days previously and that had refused to feed a second time, were inoculated into a normal monkey. The animal died of typical yellow fever on the tenth day.

In October, 1930, several hundred larvae of *C. thalassius* were collected by Dr. M. A. Barber, near New Ijora (Lagos), from a shallow, temporary ground pool which was exposed to the direct rays of the sun, and in which the water was very salty and distinctly warm to the touch. The six transmission experiments described below were performed with females reared from these larvae.

EXPERIMENT 1. On November 4th, 1930, four females engorged on an infectious monkey and were designated as Lot 377. Fifteen days later one of the two females then alive engorged upon monkey No. 2, which survived without febrile reaction and died of yellow fever after its immunity test. The same female was then inoculated into monkey No. 3, which died of yellow fever on the sixth day.

EXPERIMENT 2. On November 6th, three females engorged upon another infectious monkey, and were designated as Lot 386. Thirty-five days later two of these females were still alive and active, but they refused to engorge upon a normal monkey. They were inoculated into monkey No. 4, which died of yellow fever eight days later.

EXPERIMENTS 3, 4 and 5. On November 10th, 13th and 17th, three lots, consisting of twenty-seven, eighteen and fifty-eight females respectively, engorged on three different infectious monkeys. Eighteen, fifteen and twelve days later, infectivity tests by biting were performed with five, four and one respectively, of the females of these lots; the remainder refused to feed. All three monkeys survived the infectivity tests without febrile reaction, and died of yellow fever after their immunity tests.

EXPERIMENT 6. After the infectivity tests described in experiments 3, 4 and 5 had been performed, the three lots used in these

experiments were in effect, but not actually, combined, inasmuch as in the six subsequent infectivity tests the monkeys were exposed to the surviving mosquitoes in each of the three lots.

The details of these six tests are as follows : monkeys No. 8 and No. 10 were fed upon by three females, twenty-seven to thirty days and thirty-five to thirty-eight days respectively, after their infecting feeding. Both these monkeys died of typical yellow fever. In a third test, monkey No. 11 was fed upon by five females, forty-two to forty-nine days after their original feeding. The animal developed a severe febrile reaction, but recovered, and was later immune. In a fourth test, the five females just mentioned were killed immediately after they had engorged and were inoculated into monkey No. 12, which died of typical yellow fever.

The two remaining tests were inconclusive : monkey No. 9 was bitten by five females, twenty-five to thirty-two days after their infecting feeding ; and monkey No. 13 was inoculated with the four survivors of the combined lot, seventy-six to seventy-nine days after their infecting feeding. Both these animals survived the infectivity tests, as well as their immunity tests, without fever. It seems probable that they were immunized in the infectivity tests, although it is entirely possible that the two animals were among the small proportion of monkeys naturally resistant to the Asibi strain of virus.

Further details of these experiments may be found in the table on page 123.

Summary. Four monkeys, bitten by from one to five females that had taken their infecting blood meal twelve to eighteen days previously, survived the infectivity tests without fever and succumbed to immunity tests. On the other hand, of four monkeys that were bitten by from two to five females, after an extrinsic incubation period of from twenty-seven to forty-nine days, two died of yellow fever, one survived a febrile reaction which was interpreted as an attack of yellow fever, and one gave an inconclusive result.

Of four monkeys inoculated with from one to five females that had been infected from twelve to seventy-nine days previously, three died of typical yellow fever. The result with the fourth monkey, which was inoculated with four feeble females that had been given their infecting blood meal seventy-six to seventy-nine days previously, was inconclusive.

SUMMARY OF TRANSMISSION EXPERIMENTS

(The numbers in bold face type represent monkeys which died of yellow fever in the infectivity tests).

Experiment number	Mosquito lot number	Date of infecting feeding	Number of females which engorged	Infectivity tests							Immunity test	
				Number of days to test	Number of females alive	Number of females engorged	Number of females inoculated	Monkey number	Course	Outcome		
<i>Culex thalassius</i>												
1	377	1930 Nov. 4	4	{ 15 16	2 2	1 0	0 same 1	2 3	No fever Fever	Lived Died of yellow fever	Died of yellow fever.	
2	386	Nov. 6	3	35	2	0	2	4	Fever	Died of yellow fever		
3	384	Nov. 10	27	18	25	5	0	5	No fever	Lived	Died of yellow fever.	
4	393	Nov. 13	18	15	17	4	0	7	No fever	Lived	Died of yellow fever.	
5	390	Nov. 17	58	12	35	1	0	6	No fever	Lived	Died of yellow fever.	
6	384-A (= Lots 384, 390 and 393, combined)	Nov. 10-17	103	27-30 25-32 35-38 42-49 42-49 76-79	55 52 37 34 34 4	3 5 3 5 0 0	0 0 0 0 same 5 4	8 *9 10 11 12 *13	Fever No fever Fever Fever Fever No fever	Died of yellow fever Lived Died of yellow fever † Lived Died of yellow fever Lived	 *Survived without fever. Survived without fever. *Survived without fever.	
<i>Mansonia uniformis</i>												
7	405	Dec. 6	130	24	4	0	4	15	Fever	Died of yellow fever		
8	409	Dec. 16	618	14 21	55 4	0 0	13 4	16 17	Fever Fever	Died of yellow fever Died of yellow fever		
9	447	1931 Mar. 3	298	{ 14 15	37 37	1 0	0 same 1	18 19	No fever Fever	Lived Died of yellow fever	Died of yellow fever.	
				16 16 16	27 27 27	0 0 0	1 1 1	20 21 *22	No fever Fever No fever	Lived Died of yellow fever Lived	Died of yellow fever. *Survived without fever.	
				18	4	0	4	23	No fever	Lived	Died of yellow fever.	
10	477	June 11	142	{ 15 15 15	58 58 58	2 0 0	0 same 2 { 1 1	24 *25 26	No fever No fever Fever	Lived Lived Died of yellow fever	Died of yellow fever. *Survived without fever.	
				{ 16 16	47 47	2 0	0 same 2	27 28	No fever No fever	Lived Died of yellow fever	† High fever, but survived.	
11	480	June 17	50	16 16 16 16 16	23 23 23 23 23	0 0 0 0 0	1 1 1 1 1	29 30 *31 32 33	Fever No fever No fever No fever Fever	Died of yellow fever Lived Lived Lived Died of yellow fever	Died of yellow fever. *Survived without fever. Died of yellow fever.	
12	487	June 30	52	{ 17 17 17 17 17	36 36 36 36 36	5 0 0 0 0	0 same { 1 1 1 5 { 1 1	34 35 36 37 38 39	No fever Fever No fever Fever Fever Fever	Lived Died of yellow fever Died of yellow fever Died of yellow fever Died of yellow fever Died of yellow fever	Died of yellow fever.	
				{ 21 21	9 9	1 0	0 same 1	40 *41	No fever No fever	Lived †	Died of yellow fever.	
13	491	July 6		{ 17 17 17 17 17	8 8 8 8 8	1 0 0 0 0	0 same 1 1 1 1	42 43 44 45 46	No fever Fever Fever Fever Fever	Lived † Lived Died of yellow fever † Lived Died of yellow fever	Died of yellow fever. Survived without fever. Survived without fever.	

* Inconclusive result.

† Interpreted as an attack of yellow fever.

‡ Died of dysentery before it was given its immunity test.

Discussion. The fact that the transmission of yellow fever by bites of *Culex thalassius* was obtained only after an extrinsic incubation period of twenty-seven days, indicates that this mosquito is an inefficient vector in comparison with *Aedes aegypti*. On the other hand, the density of the species is known to be very great at certain places in a narrow zone along the coast, and this may compensate for its relative inefficiency as a vector, and may make it of some importance in the epidemiology of yellow fever.

MANSONIA UNIFORMIS

Notes on Life Habits. According to Edwards (1923-24), *Mansonia uniformis* is found throughout the Ethiopian, Oriental and Australasian zoo-geographical regions. Macfie and Ingram (1916-17), have studied its distribution in the Gold Coast, and report that it is abundant in a relatively narrow zone along the coast, diminishes in numbers in the forest belt, and becomes a rare species in the far inland semi-arid regions. Its distribution in Nigeria appears to be similar to that in the Gold Coast. It is abundant in parts of the environs of Lagos, but is not found in the city itself (Beeuwkes, Kerr and Weathersbee). It is, the author believes, absent from the large interior cities of Ibadan, Zaria and Kano, though Edwards (1913-14) reports it from six widely separated small places in the Northern Provinces. In the vicinity of Lagos, during the height of the dry season, the author took large numbers of adults near some extensive marshes, the waters in which were then slowly receding. With the approach of the rainy season, its numbers diminished very markedly, and only began to increase when the marshes filled again with the rains.

The breeding places of this species are obviously swamps and marshes, but it is not found on *Pistia stratiotes*.* In spite of its occurrence over so very wide a region, its immature stages appear to have been taken only once—at Singapore (Edwards and Given, 1927-28).

The author has found that *M. uniformis* is active throughout the night, beginning at sunset. It is a vicious biter of man in the open,

*J. Bonne-Wepster (1930) records finding the larvae of *M. uniformis* around Batavia attached to a species of *Pistia* in a shallow fresh water swamp.—EDD.

and it readily enters houses to feed. There is little doubt that the females prefer human blood, and that, in nature, they feed repeatedly upon man and live long enough for an ordinary extrinsic incubation period to be completed.

Transmission Experiments. The females used in these experiments were all captured at night in the vicinity of Lagos, with human bait. This was necessary because larvae and pupae from which adults might be reared could not be found. All the captured females that contained a visible amount of blood were discarded, and only unfed females were exposed to infectious monkeys. *M. uniformis* was found to be difficult to work with in captivity because of the early death of a large proportion of the engorged females and their unwillingness to feed a second time. Both these characteristics seem to have some connection with the reluctance of the females to oviposit in captivity. Aside from six attempts to secure transmission, which were completely frustrated by the early death of the engorged females, the results of seven experiments are set forth in detail in the table. For the sake of brevity, the protocols of these experiments are omitted.

The reactions of the monkeys in the various infectivity and immunity tests were interpreted in the same way as were similar reactions in the monkeys used to test *Culex thalassius*.

The early high mortality of the mosquitoes used in the first three experiments, which were performed during the hot dry season, as compared with the much lower mortality in the later experiments carried out during the cool rainy season, is noteworthy.

In the last five experiments, immediately after the *M. uniformis* females had engorged upon infectious monkeys, control lots of *Aedes aegypti* were allowed to engorge upon the same monkey. These control lots were all highly infective.

Summary. The six monkeys, that were fed upon by from one to five proven infected *M. uniformis* females fourteen to twenty-one days after their infecting feeding, survived the infectivity tests without any febrile reaction. Five of these monkeys died of yellow fever after their immunity tests, and the sixth gave an inconclusive result.

Five monkeys were inoculated with from two to thirteen females which had engorged upon six different infectious monkeys from

fourteen to twenty-four days previously. Four of these monkeys died of yellow fever, but the fifth survived the infectivity test without any febrile reaction and died of yellow fever after its immunity test.

Twenty-one monkeys were inoculated with single females which had engorged upon infectious monkeys. In the infectivity tests, twelve of these monkeys died of yellow fever, and two others survived severe febrile reactions, which were interpreted as manifestations of this disease. These two monkeys survived their immunity tests without fever. One of the remaining seven monkeys died of dysentery before it could be given its immunity test. Of the six other monkeys which were so tested, three died of yellow fever, and three survived without febrile reactions. The results with these three monkeys, and with the one that died of dysentery, were considered to be inconclusive.

Discussion. The transmission experiments show that, under the conditions provided, small numbers of *M. uniformis* are not capable of transferring lethal amounts of yellow fever virus by their bites after an extrinsic incubation period ranging up to twenty-one days—the longest period after which they could be induced to feed a second time. Conditions in the bodies of a small proportion of these mosquitoes seem to be deleterious to the virus, but a large proportion (fourteen out of seventeen) are capable of retaining the virus in amounts lethal to monkeys for at least fifteen to seventeen days. Furthermore, some females are able to retain the virus for twenty-four days, and it seems probable that most *M. uniformis* females can retain the virus throughout their lives. It was not found possible to keep these mosquitoes alive in captivity long enough to demonstrate conclusively whether or not they are able to transmit yellow fever by their bites; but the long extrinsic incubation period that appears to be essential would prevent this species from being an efficient vector of the disease, as compared with *Aedes aegypti*.

The inconclusive nature of these negative experiments makes it wise to consider *M. uniformis* as a possible vector, of importance only where and when it is very prevalent. Its possible importance is increased by the fact that it is very likely to be confused with its congener, *M. africana* (= *Taeniorhynchus africanus*), which has been shown experimentally to be a vector, though not an efficient one.

CONCLUSIONS

Culex (Culex) thalassius is capable of transmitting experimental yellow fever by its bite, after a long extrinsic incubation period, and it may be of some importance in the epidemiology of yellow fever at the places along the West African coast where its density is great.

Mansonia (Mansonioides) uniformis females appear to be unable to transfer the virus of yellow fever by their bites after an extrinsic incubation period of twenty-one days or less, but they are able to retain the virus, probably throughout their lives. This species cannot be entirely ruled out as a possible factor of some importance in the epidemiology of yellow fever in the places where it is abundant.

ACKNOWLEDGMENTS

The writer is greatly indebted to Mr. F. W. Edwards, of the British Museum (Natural History), who examined representative specimens of *Culex thalassius* and *Mansonia uniformis* and confirmed the author's identification of these species; and to the director of the West African Yellow Fever Commission, Dr. Henry Beeuwkes, and the members of the staff of the Commission, all of whom have assisted in many ways in this work.

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THE INFECTION OF A GUINEA-PIG WITH *TRYPANOSOMA CONGOLENSE* AND *T. GAMBIENSE* FROM THE BITE OF *GLOSSINA LONGIPALPIS*

BY

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I. INTRODUCTION

During the first six months of 1931, the writer was engaged in an ecological study of *Glossina longipalpis*, at Takoradi, in the Gold Coast. In studying the trypanosomal infection of the fly by Lloyd and Johnson's method (Lloyd and Johnson, 1923) of dissecting and examining proboscis, salivary glands and mid-gut, heavy infections of trypanosomes were found in the wild flies. Of the flies examined in May and June, 1931, 20 per cent. showed mature *T. congolense* infections and 4 per cent. *T. vivax* infections.

Experiments were then undertaken of feeding wild infected flies on guinea-pigs in an attempt to infect the animals with the trypanosomes mentioned above; and these resulted, in one case, in the successful establishment of a mixed infection of *T. gambiense* and *T. congolense* in a guinea-pig. This mixed infection was then passed through a number of experimental animals, and although the number of experiments undertaken was not sufficiently large to warrant any definite conclusions being drawn, yet it is hoped that the results are of sufficient interest to justify their publication.

II. THE EXPERIMENTS TO INFECT GUINEA-PIGS

The guinea-pigs used in the experiments were bred in the laboratory in fly-proof shelters, and were always examined for trypanosomes in the blood before use. The rats used were the common bush rat, *Cricetomys gambianus*, and the black rat, *Mus rattus*; these were subjected to daily blood examination for ten or fourteen days before the experiment to ascertain their freedom from parasites.

The procedure in the experiments was to feed wild flies, one at a time, on the experimental animal, and then dissect and examine the flies for mature trypanosome infections. When it was found that an infected fly had been fed, the experiment was discontinued and the animal placed under observation by the daily examination of fresh blood.

In some cases an intraperitoneal injection of olive oil was given to the animal three days after the fly had been fed upon it. This has been found by Saunders (1929) to increase the susceptibility of the animal to trypanosome infection.

Six guinea-pigs had flies with heavy mature infections of *T. congolense* fed upon them. Three of these had subsequent injections of olive oil. One of the guinea-pigs (guinea-pig 3), to which no olive oil had been given, developed a mixed infection of *T. gambiense* and *T. congolense* after an incubation period of thirty-four days. Altogether, five flies had been fed upon this guinea-pig, the last of which was found infected with *T. congolense*. The *T. gambiense* infection must have been missed in the examinations. The course of the infection will be described below.

Further attempts were made to infect three guinea-pigs by the injection of a suspension of teased-up proboscis of *T. congolense* infected flies in saline. To two of these animals olive oil was subsequently given. The result in every case was negative.

III. THE COURSE OF THE INFECTION IN GUINEA-PIGS AND RATS

The infecting feed in guinea-pig 3 was on April 27th, 1931. The animal's blood was examined daily, and the first infection was seen on June 1st, 1931, after thirty-four days' incubation period. Only one trypanosome was seen in a fresh blood preparation, and the infection remained thus for two weeks. On June 8th, 1931, 1 c.c. of olive oil was injected intraperitoneally to produce relapse, without effect. On June 13th, 1931, 2 c.c. of olive oil were given, and on June 16th, 1931, two or three trypanosomes were seen on a slide, and by the 20th of the month parasites were quite numerous. A thin blood film showed the presence of both *T. gambiense* and *T. congolense*. In 100 fields, using a 1/6 in. objective and $\times 10$ eyepiece, fifty

T. gambiense and seventeen *T. congolense* were counted. Thin films were taken every three days and examined, and the course of the infection is represented graphically in Chart I. (Throughout this, and subsequent examinations, a standard count of 100 or more fields was made with the 1/6 in. objective and $\times 10$ eyepiece, the slide being examined *across* and not *along* its main axis, in order that the heavier concentration of parasites at the edges of the film might be included, as well as the more dispersed parasites in the centre.) On June 26th, very few trypanosomes could be seen, but they had increased again by the 29th. Now, however, *T. congolense* was in the majority by about 2 to 1. A very great increase in *T. congolense* occurred on July 11th, while *T. gambiense*, except for temporary day to day fluctuations, remained at much the same intensity of infection. From July 11th to August 10th there was a fairly stable period in the infection of *T. congolense*, followed by another considerable increase in numbers, so that at the end of the month this species was swarming in the blood. This was accompanied by a slight increase in *T. gambiense*, which was now showing large numbers of stumpy forms, the proportion of long to short forms being about 3 to 1. The guinea-pig was now very weak and exhibiting acute anaemia, and died on August 29th, 1931, a hundred and twenty-four days after the infecting bite of *Glossina*, the infection having lasted ninety days. Apparently *T. congolense* was the more influential in bringing about death.

During the period of the infection from July 11th up to the death of the animal, numbers of very broad, pale staining *T. congolense* appeared. These were apparently degenerate trypanosomes, as every stage was seen, from forms longer and broader than the typical, but with a clearly defined, though paler staining nucleus, to those in which the trypanosomes were semi-circular or globose in shape, 12 to 15 μ long and 6 or 7 μ broad, the side remote from the undulating membrane ragged or ill-defined, and the parabasal body and the nucleus scarcely discernible, the latter a mere shadow in the very faintly staining cytoplasm. Intermediate forms showed the nucleus becoming granular, ill-defined in outline, and apparently breaking up, while the parabasal body and undulating membrane were still sharply stained and conspicuous. In subsequent passages of this strain in guinea-pigs, the occurrence of these forms was always noticed in the

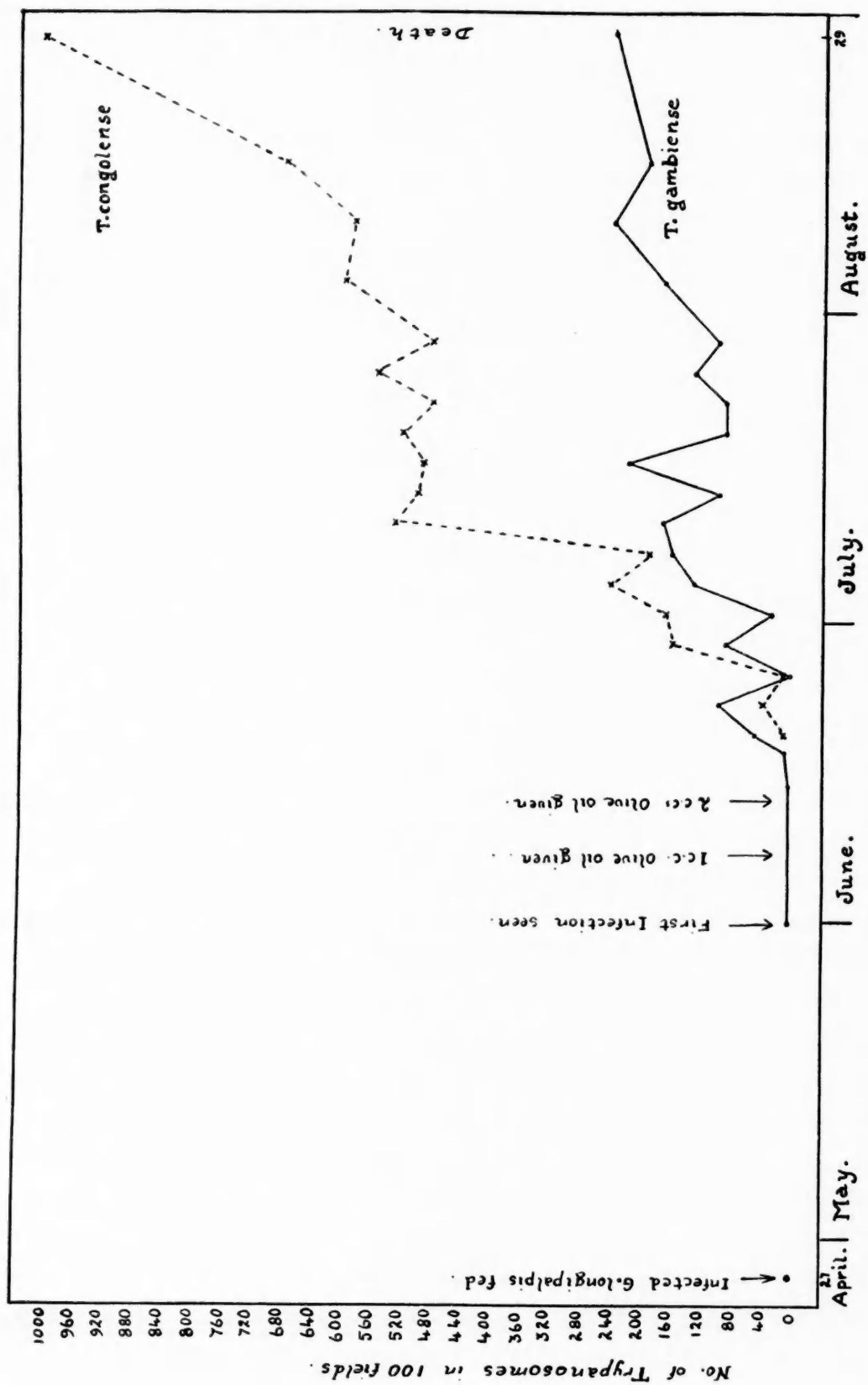


CHART I. Initial infection of a guinea-pig with a mixed strain of *T. gambiense* and *T. congolense* from a wild *Glossina*.

later stages of the infection when *T. congolense* was very numerous. In the passage of the strain in rats, these forms did not occur, but a similar type of very broad, pale, degenerating *T. gambiense* was seen. In the rat *T. gambiense* was swarming at death, *T. congolense* being present in comparatively small numbers.

Inoculations from guinea-pigs were made into other guinea-pigs and several rats. Guinea-pig 7 was inoculated with 0.25 c.c. of blood on June 18th, 1931, before the trypanosomes were very numerous. The first infection was seen on June 26th, 1931, 1 c.c. of olive oil having been given on the previous day. For the first six days of the infection, only *T. gambiense* could be seen, and in small numbers, 5 to 10 in 100 fields. On the seventh day, *T. congolense* appeared and increased rapidly, at the same time *T. gambiense* showed a slight decrease. After this the infection took a course similar to that in guinea-pig 3, but of shorter duration. Trypanosomes were abundant on the fortieth day after inoculation, 158 *T. gambiense* and 704 *T. congolense* in 100 fields. Trypanosomes were swarming on the sixtieth day, and on the sixty-fourth day the guinea-pig died, showing a predominance of *T. congolense* to *T. gambiense* by about 4 to 1.

In rats the infection ran a very much shorter course, and the strain was found to be far more virulent to the local bush rat, *Cricetomys gambianus*, than to *Mus rattus*. Apparently *T. gambiense* was the more pathogenic organism to rats, being predominant throughout. *T. congolense* only appeared in the later stages of the infection when the former species was swarming.

In rat 3 (*C. gambianus*), inoculated from guinea-pig 3 on June 18th, 1931, infection appeared in four days; on the fifth day, *T. gambiense* was very numerous, about 16 per field; on the eighth day, a few *T. congolense* were seen on a slide, *T. gambiense* now appeared 25 or more per field; the animal died on the thirteenth day, *T. gambiense* now occurring 30 to 40 per field, *T. congolense* 5 or 6 per field. *T. gambiense* was showing long and short forms in about equal proportions, and also occasional degenerate forms, very broad and short, with very pale staining cytoplasm and disintegrated nucleus.

In *M. rattus* the infection usually appeared on the fifth day; at first *T. gambiense* only showed, and the infection did not become heavy for three or four days. This was followed by a sharp rise in

numbers, and the appearance of *T. congolense* now took place. Death resulted in twenty to twenty-four days. Chart II shows graphically the course of infection in rat 9 (*M. rattus*), inoculated with 0.25 c.c. of blood from guinea-pig 3 on August 20th, 1931.

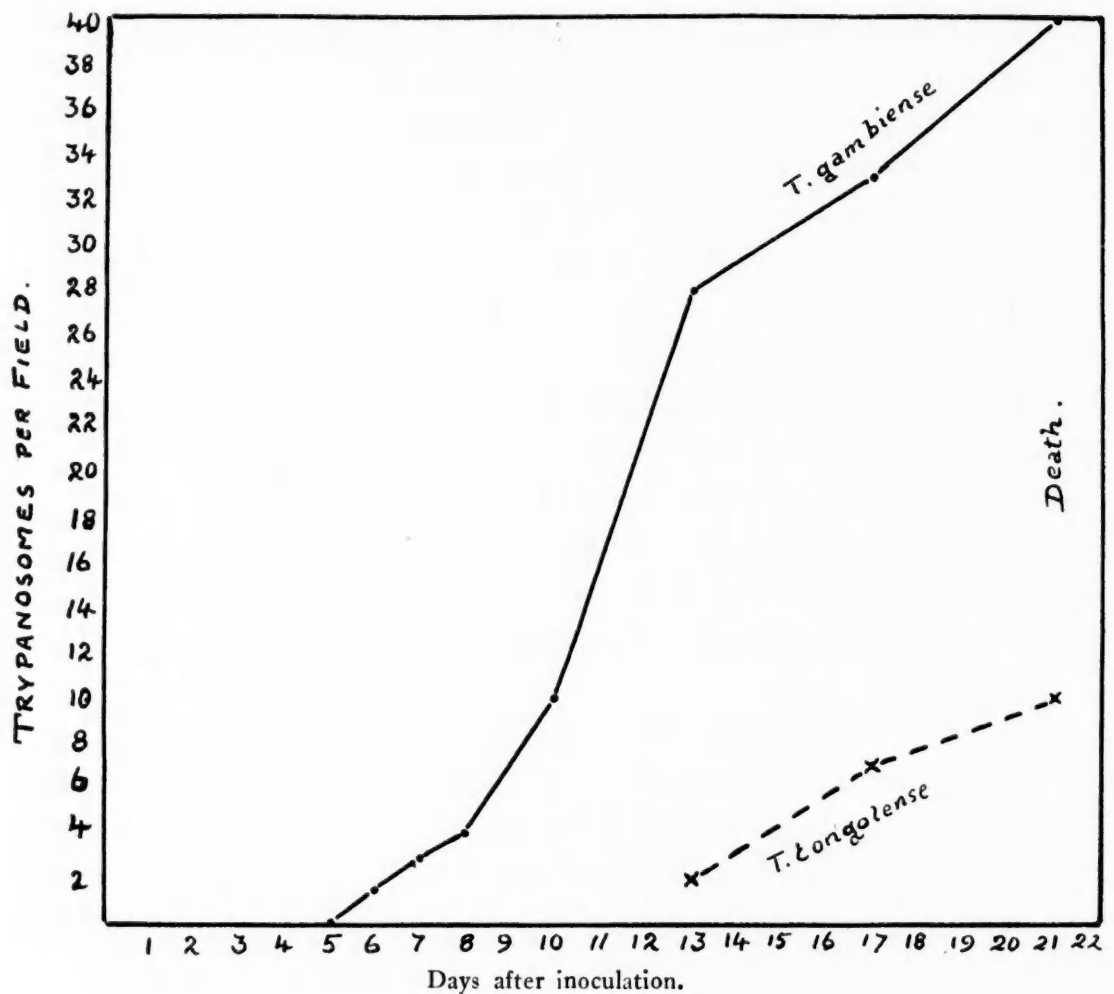


CHART II. Infection in *Mus rattus*.

The case of another *M. rattus*, rat 4, inoculated with the same strain a few weeks earlier, is interesting. This rat developed a septic tail a week after the inoculation, and at the same time the infection of *T. gambiense* relapsed, from about 4 per field to a very light infection of a few parasites only in a slide. The trypanosomes increased again on the fifteenth day, but again relapsed. From the twenty-first day there was a steady rise in infection, and *T. congolense* appeared on the twenty-fourth day. The rat died on the twenty-seventh day but showed an infection considerably lighter than in the case of the other experimental rats. Chart III illustrates the course

of this infection. Saunders (1929) has observed a similar inhibiting action of sepsis on the development of trypanosomes in rats.

In subsequent inoculations into guinea-pigs and rats this mixed infection followed much the same course, but tended to become more virulent to the animals. Rat 7 (*C. gambianus*), inoculated from guinea-pig 7 on July 22nd, 1931, showed the first infection on the fourth day and died on the tenth day. Rat 8 (*C. gambianus*), inoculated from guinea-pig 7 on August 20th, 1931, became infected in three days and died in six days. Guinea-pig 11, inoculated from rat 8 on August 26th, 1931 (*T. gambiense* swarming and *T. congolense* scarce), showed infection with *T. congolense* on the fifth day and died on the

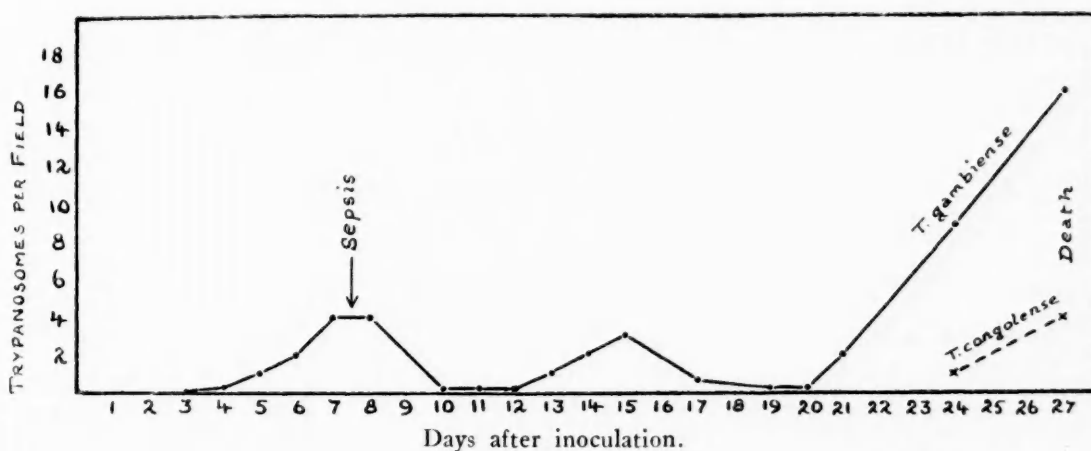


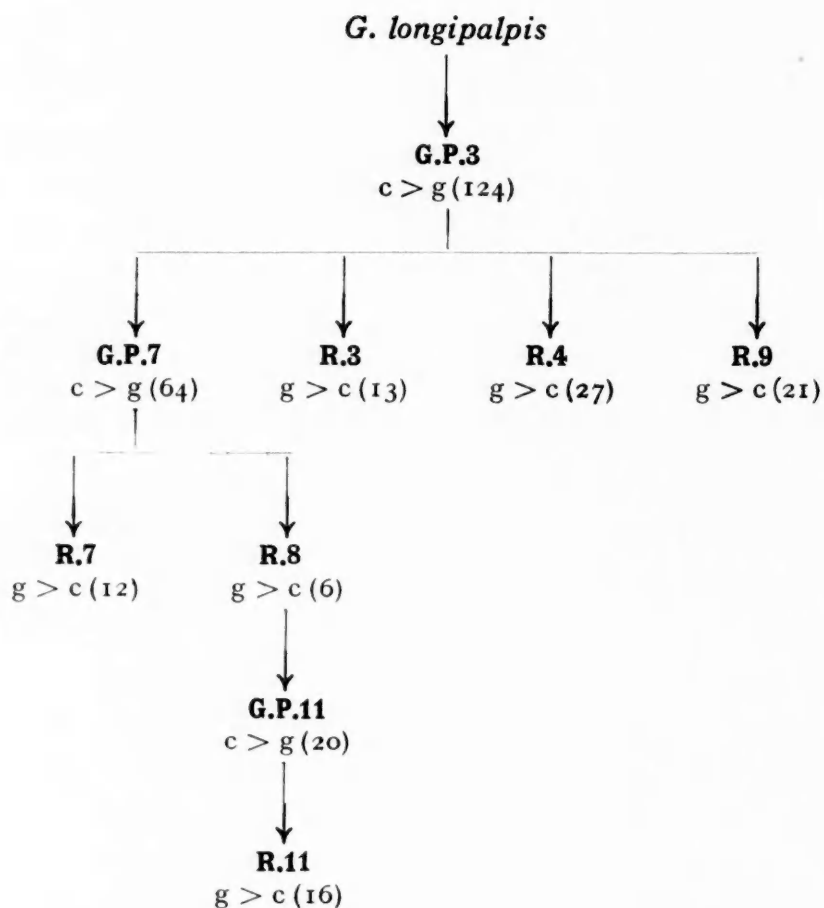
CHART III. Progress of infection in *M. rattus* which developed a septic tail.

twentieth day, with a swarming *T. congolense* and scanty *T. gambiense* infection. Rat 11 (*M. rattus*), inoculated from guinea-pig 11 on September 10th, 1931, developed the infection in seven days, but died on the sixteenth day, with *T. gambiense* 30 in the field, *T. congolense* 4 to 5 per field.

Fig. 1 depicts the passage of this mixed trypanosome infection as described above. For the sake of clearness, only those animals that have been mentioned are shown in the table.

The writer had started a further series of experiments on feeding *T. congolense* infected *Glossinae* on guinea-pigs already infected with *T. gambiense*. These had to be abandoned, however, owing to the writer's proceeding on leave and the closing down of the tsetse fly research work.

FIG. 1



G.P. = Guinea-pig. **R.** = Rat.

c > g = predominance of *T. congolense*.

g > c = predominance of *T. gambiense*.

The figures in brackets indicate the number of days' duration of the infection.

IV. DISCUSSION

It seems significant that, out of a series of attempts to establish *T. congolense* in guinea-pigs, both by exposing the animals to the bites of infected flies and also by the injection of dissected and teased-up probosces, the only success was obtained when another trypanosome (*T. gambiense*) was present as well. In the original animal, *T. gambiense* was the first to appear in the peripheral blood, and this proved to be the case in sub-inoculated animals. The subsequent behaviour of the sub-inoculated animals, however, differed according

to the species of the host. This raises the important question of the susceptibility of any given species of host to any given trypanosome, and whether this susceptibility may be modified by a double infection.

In guinea-pigs, the course of the infection was as follows. First the appearance of *T. gambiense*, followed shortly by the appearance of *T. congolense*, which was usually coincident with a slight relapse of the *T. gambiense* infection. From this point *T. congolense* invariably increased the more rapidly, swamping the first infection and finally killing the animal after a more or less prolonged infection.

In rats (*C. gambianus*), *T. gambiense* appeared in three or four days, increased immediately, and killed the animal in six to twelve days. *T. congolense* appeared only in the last stage of the infection when *T. gambiense* was swarming. In *M. rattus*, there was essentially the same course but the disease was prolonged. In one individual *M. rattus*, the course of the infection was definitely modified when the animal developed a septic tail, the trypanosome strain relapsing twice and the rat finally dying with a much lighter infection than that killing a healthy rat.

From this evidence one might infer that there is definite influence of one parasite on the other—of *T. gambiense* sensitizing the animal for heavy *T. congolense* infection; or of the presence of a bacterium inhibiting the development of a protozoan. This latter certainly might be brought about in two ways: the direct effect of the bacterium or its waste products on the trypanosome; or the indirect effect, the blood of the unhealthy rat, offering a less favourable pabulum for the trypanosome. If we regard the predisposing effect of olive oil towards trypanosome development as due to an increase in the sugar content of the blood, then this indirect effect seems the more likely.

On the other hand, one might attempt to explain this differential effect of the two species of trypanosome on their hosts in terms of the time factor. The rat (highly susceptible to trypanosomiasis) is killed by the more quickly developing *T. gambiense* before *T. congolense* has really got a footing. In the guinea-pig (less susceptible to trypanosomiasis), the prolonged course of infection allows the necessary time for the full development of *T. congolense*. This argument, however, involves such a number of unproven hypotheses that it must not be taken as having more than a suggestive value.

We are still left with our most important question unanswered—the question of the powers of one trypanosome modifying the susceptibility of an animal towards another trypanosome—and the answer to this will only be forthcoming after further experimental work.

SUMMARY

1. A mixed infection of *T. gambiense* and *T. congolense* was established in a guinea-pig from the bite of a wild *Glossina longipalpis*.

2. In the first and subsequent passages through guinea-pigs, *T. congolense* was predominant in the late stages of the infection and apparently was responsible for death; *T. gambiense* was usually the first trypanosome to appear, but remained in moderate numbers in peripheral blood.

3. The infection was more virulent to *Cricetomys gambianus* than to *Mus rattus*; in both rats *T. congolense* was always swarming in the blood at death, *T. gambiense* only appearing in moderate numbers in the latter stages of the infection.

4. The infection increased in virulence towards both rats and guinea-pigs in successive passages by injection.

5. In a *M. rattus* that developed a septic tail after infection, the *T. gambiense* strain relapsed, but subsequently killed the rat.

ACKNOWLEDGMENT

The writer wishes to express his thanks to Professor J. G. Thomson, of the London School of Hygiene and Tropical Medicine, for his very great kindness in helping and advising during the writing of this paper.

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PLASMODIUM OVALE STEPHENS :
PASSAGE OF THE PARASITE THROUGH
MOSQUITOES AND SUCCESSFUL TRANS-
MISSION BY THEIR BITES

BY

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(Received for publication 15 March, 1932)

In 1922, Stephens reported that the morphological characters of the asexual forms of a malaria parasite, which he had studied in the blood of a patient from East Africa, were different from those of any usually accepted species, and he proposed to establish the parasite as a new species with the name *Plasmodium ovale*. In 1927, in collaboration with D. Uvedale Owen, he confirmed and extended the description from the study of a case infected in Nigeria. In 1930, Warrington Yorke and D. U. Owen studied another case from Nigeria, and, by utilising this patient's blood in the practice of malariatherapy, they were able to show that the peculiar morphological features of the asexual stages of the parasite remain constant when it is passed from person to person by direct blood inoculation.

Our study of the parasite dates from October, 1931, when Professor Warrington Yorke kindly sent us a tube of citrated blood taken from a patient whom he had infected by direct inoculation with the blood of a patient from the Belgian Congo. We received the blood on October 29th, and inoculated it intramuscularly into a patient who was awaiting treatment for general paralysis and had not previously been infected with any form of malaria. The patient developed the malarial attack after an incubation period of six days, and we were able in this case to observe that the morphological characters of the asexual parasites corresponded with the description

of *P. ovale* given by previous workers. Thereafter we took steps to maintain the strain continuously at Horton by direct blood inoculation from patient to patient, and we also used it occasionally for malaria-therapy at other hospitals. In all, fourteen patients with general paralysis were given a course of malariatherapy by direct inoculation with this parasite during the three months November to January, and in all of them the course of the fever and the morphological characters of the asexual parasites were the same.

At Horton, our chief object was to study the sexual forms of the parasite, to ascertain if they would undergo development in the mosquito, and, if that were so, to infect some new cases by the bites of infected insects in order to see if the morphology of the parasite would present the same characteristic features as in the original infecting case. A primary difficulty encountered in this enquiry was that cases infected with *P. ovale* by blood inoculation tend to recover without quinine treatment before the appearance of many gametocytes in the peripheral blood. And when we tried our plan of aborting the attack by giving a single dose of quinine, in the expectation that gametocytes would appear in greater numbers in the recrudescence, we found that the single dose cured the case. For this reason it was not until the end of December that (in our fourth case at Horton) we were successful in demonstrating the exflagellation of male gametocytes in moist films. The patient in whom these forms of the parasite were found was inoculated first on December 2nd and 3rd, but reacted only with temperatures of 100° on the 15th and 17th (parasites 1 in 200 fields), and then appeared to be about to recover. He was therefore re-inoculated from another patient on the 20th, and this re-infection caused a typical attack to begin on the 23rd. Gametocytes were present on the 28th, and exflagellating forms were demonstrated on the 30th. On January 1st, the count of gametocytes was twelve males and seventy-two females per c.mm. of blood. A batch of forty *Anopheles maculipennis* was fed on the case from December 29th to January 1st, and incubated at 25° C. Several oökinetes were found in films of blood prepared from the stomach contents of some mosquitoes of this batch, but the results of later dissections for oöcysts were disappointing, for only one small oöcyst was found at the examination of twenty-two insects between January 2nd and 4th.

Case 5 at Horton was the next in which gametocytes became moderately numerous. The infection in this case was by the intramuscular inoculation of 10 c.c. of blood from the case just described. Gametocytes were found on the thirteenth day after the beginning of fever, and exflagellating forms were demonstrated two days later. The counts of exflagellating forms and of female forms in moist chamber films on this day and later were as follows :—

Date	Per 100 leucocytes		Per cubic millimetre	
	Males (exflagellating)	Females	Males (exflagellating)	Females
27.1.32	3	5	210	350
28.1.32	2	5	140	350
29.1.32	1	3	70	210
30.1.32	1 per 200	4	35	280
31.1.32	Very rare	Very rare
1.2.32	"	"
2.2.32	"	"

A batch of a hundred *A. maculipennis* was fed daily on the patient from January 27th to 31st, and incubated at 25° C. The patient's temperature chart during this period is reproduced below :—

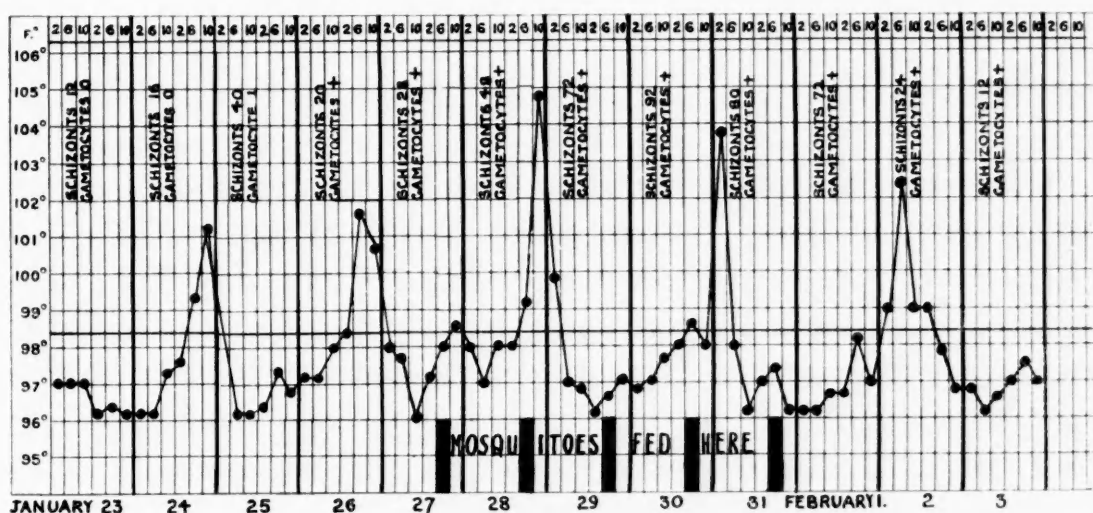


CHART I. The infecting case.

After the batch had been fed during those five days on the infecting case, feeding was continued on two patients who had never had malaria. Some of the insects which died each day were dissected, with the following results :—

Date and day after first feed	Number dissected	Number with oöcysts in the stomach	Number of oöcysts found in each	Number with sporozoites in the glands
Jan. 30th (3rd day)	2	1	3	Not examined
„ 31st (4th „)	3	2	1 and 5	„
Feb. 1st (5th „)	3	2	3 and 17	„
„ 2nd (6th „)	3	2	4 and 42	„
„ 3rd (7th „)	2	2	2 and 5	„
„ 4th (8th „)	4	1	1	„
„ 7th (11th „)	2	1	1	○
„ 8th (12th „)	2	1	1	○
„ 10th (14th „)	2	○	...	○
„ 11th (15th „)	1	○	...	○
„ 12th (16th „)	2	Not examined	...	*2
„ 14th (18th „)	1	„	...	1
„ 16th (20th „)	10	„	...	†5

* On the morning of dissection one of these mosquitoes had fed on one of the patients who was awaiting treatment.

† Yesterday one of these mosquitoes had fed on the other patient who was awaiting treatment.

Thus, of the thirty-seven mosquitoes dissected, twenty (54 per cent.) were infected. The infections, however, were light. The number of oöcysts on each stomach varied from one to forty-two, with an average of seven, and at one period it appeared as if a large proportion of them would fail to complete their development to the sporozoite stage. The gland infections of the five positive insects dissected on the last day were scanty ; in one specimen only eight sporozoites could be found after prolonged examination. It will be seen from the tabular statement that the cycle to the stage of

sporozoites in the glands occupied sixteen days at 25° C. This is very different from our usual result (ten days) with *P. vivax* in the same species of mosquito at that temperature. On the other hand, it is a better result than we have yet obtained with *P. malariae*.

TRANSMISSION BY INFECTED INSECTS

As already mentioned, two patients, who had not previously had malaria, were selected as recipients of the infection by mosquito bites. It was known that one of them was bitten by an infective mosquito on February 12th, and the other on the 16th. Fearing, however, that, as the glands of those insects were only lightly infected, one or both of the infections might fail to take, or might remain latent, we decided on February 16th to select two additional patients and to infect them by the intravenous injection of sporozoites from the glands of the five insects which were found to be positive on that day. No new patients awaiting treatment being available, we selected for the purpose two patients who had already gone through a course of malariatherapy with *P. ovale* by direct blood inoculation. They were our first and fourth cases in the *ovale* series at Horton; they had completed the primary course ten weeks and five weeks respectively prior to the date of receiving the new infection.

As it turned out, this precaution was not necessary, because both the cases bitten by infected insects developed the malarial attack, one with an incubation period of fourteen days, the other of fifteen days. Both cases re-infected by the intravenous injection of sporozoites also started a new attack.

Thus there were available four cases from which to ascertain the effect on morphological characters and on clinical signs of passing *P. ovale* through the mosquito. Taking clinical signs first, the temperature chart (page 144) of the patient who was bitten by one or more infected mosquitoes on February 12th can be compared with the chart already given of the patient from whom those mosquitoes received the infection between January 27th and 31st.

It will be seen that in the developed stage the febrile paroxysms are of the 'single tertian' type with an interval of forty-eight hours between the onset of each paroxysm, as was the case in the attack of the patient by whom the mosquitoes were infected. Blood

films were examined and the numbers of each stage of the parasite counted per 100 leucocytes every four hours over a period of forty-eight hours from March 5th to 7th, and the results showed conclusively that the asexual cycle has a forty-eight hour periodicity of sporulation corresponding with the fever curve.

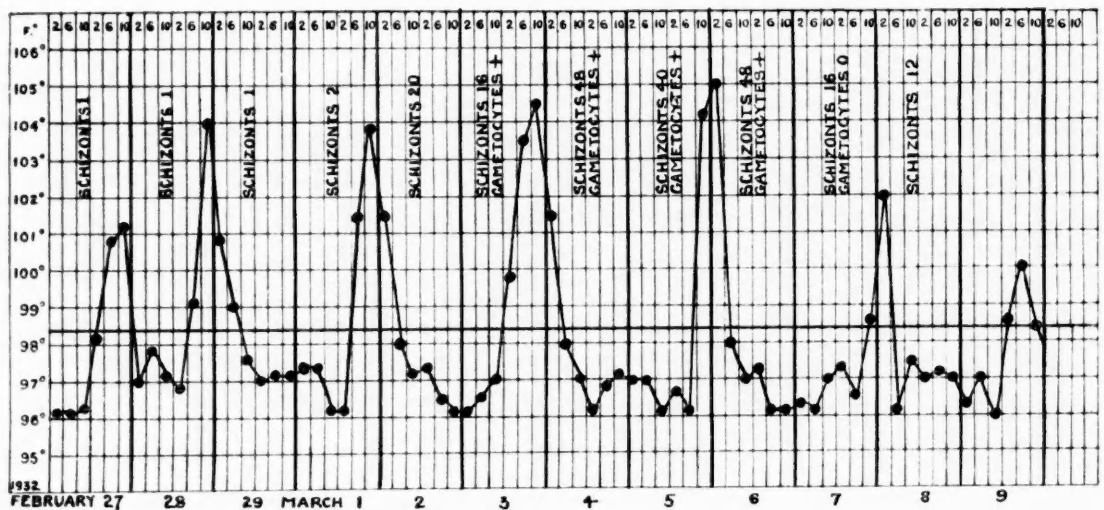


CHART II. The case infected by the mosquitoes which had fed on the infecting case (Chart I).

The other patient who was bitten by the same infected mosquitoes showed in the developed stage of the attack a 'double tertian' type of fever with a febrile paroxysm every day. In this case, by the examination of blood films every four hours over the same period as in the first case, we were able to prove that the daily paroxysms were due to the presence of two groups of the parasite, one completing its forty-eight asexual cycle on March 5th, 7th and 9th, the other on the 6th, 8th and 10th.

During these examinations we ascertained that the morphological characters of the parasite in its asexual stage, as well as its effect on the red corpuscles in which it is situated, were precisely the same as in the original infecting case, and that the description of these features given by Stephens (1922 and 1927), and confirmed by Warrington Yorke and Owen (1930), was applicable to them in all essential respects. By these examinations we were able, also, to confirm the correctness of the conclusion reached by Stephens that 'the morphological characters of the asexual forms of the parasite are different from those of any usually accepted species.' Moreover, from our examinations of the developmental stages of the sexual

forms of the parasite in the mosquito, we are able to say that the arrangement of the pigment in the stage of young oöcysts is so different from that of any of the species hitherto known as to enable the parasite to be identified without difficulty at this stage of its life cycle. We shall take an early opportunity of describing and illustrating those and later stages of the mosquito cycle, as well as the sexual stages of the parasite in the human host which were not studied by previous observers. In the meantime we can say definitely that there remains no doubt that *P. ovale* is a separate species, and that its morphological characters in the human and insect hosts are characteristic and constant.

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A SIMPLE APPARATUS FOR BREEDING FLEAS

BY

E. P. HICKS

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Sierra Leone)

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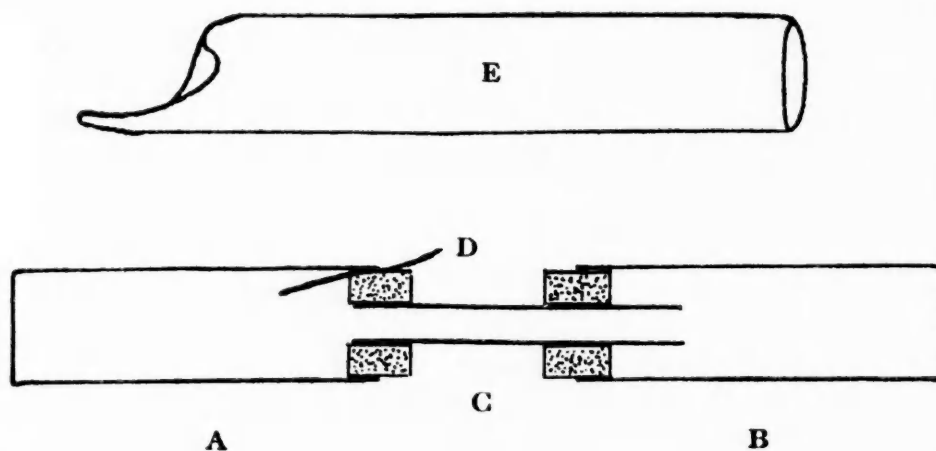
When breeding flea larvae, it is convenient to keep them in a flat dish with a loose cover, such as a petri dish, so that they may easily be observed through a dissecting microscope. But when they are about to pupate, they must be confined in a securely closed vessel to prevent the escape of the adults. For this a corked or stoppered bottle is not altogether suitable. The period during which adults of a given batch of larvae are emerging from their pupae may be as long as one or two weeks. It is therefore desirable to have some means of removing the adults each day as they emerge, without destroying the rest of the culture; for if they are left in the vessel too long, they die and become dirty, shrivelled and mouldy.

The figure shows an apparatus which was devised for dealing with *Tunga penetrans*. Two glass containers, A and B, are fitted with bored corks through which passes the glass tube C. One end of the tube is flush with the inner surface of the cork in A, the other end projects into the interior of B. A slip of damp blotting paper, D, may be inserted between the cork and side of A to raise the humidity, if necessary. In dealing with a flea as small as *T. penetrans* it is as well to cover the corks with wax, to prevent the escape of the fleas through some unnoticed crevice. A cylinder of brown paper, E, is made to fit the apparatus from the base of A to the cork of B. This is loose and can be slipped on and off.

The size of the containers in use is 75×22 mm., and the bore of the tube is 8 mm.; but presumably the dimensions are not of great importance.

The culture of the larvae, with the material in which it is breeding, is placed in A, the apparatus is closed, and the brown paper cylinder

slipped over A. As the adults emerge inside the dark container, they make for the light and find themselves in B. When sufficient adults have collected, the container B, complete with its cork, is slipped off the tube C, cotton wool plugs are fitted into the tube C and the cork of B, and the fleas are killed, if desired, by blowing chloroform vapour into B by means of a capillary pipette. When



the fleas have been removed, B may again be fitted on to the tube C, after allowing a short time for the chloroform vapour to disperse. During the time that B is separated from the apparatus, the brown paper cover should be removed from A, so that the fleas may not be encouraged to wander down the tube.

This apparatus made it possible to observe that, of a culture of *Tunga penetrans* bred from eggs laid by a number of fleas on the same day, the females emerged before the males, as shown below in the table. This is contrary to the sequence observed among some other insects, for example mosquitoes.

TABLE

Showing the days on which the males and females of a culture of *Tunga penetrans* emerged from the pupae.

Day of emergence	Male	Female
1 to 3	0	50
4	9	4
5 to 10	83	0

YAWS AND SYPHILIS IN CALABAR, SOUTHERN NIGERIA: AN ANALYSIS OF 5,000 SACHS-GEORGI TESTS

BY

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(Received for publication 3 May, 1932)

In parts of the southern provinces of Nigeria, yaws is well known to be a considerable factor in the morbidity of the population ; and during the time that the author was stationed in Calabar he endeavoured to assess the extent to which the children were infected. To this end, two thousand six hundred children between the ages of four and sixteen years were examined, and, for purposes of comparison, a series of two thousand four hundred adults was examined at the same time. One thousand, six hundred and ninety children were obtained from the schools in Calabar* ; and the remaining nine hundred and ten children, together with the two thousand four hundred adults, were taken at random from the out-patient departments and wards of St. Margaret's Hospital.

TECHNIQUE

In each case 5 to 10 c.c. of blood were drawn from one of the veins of the arm, and the serum submitted to the Sachs-Georgi test. The bloods were taken in the morning and the tests were put up in the afternoon, the results being read the following morning after the tubes had been rather over eighteen hours in the water bath at 37° C. The antigen used was that supplied by Messrs. Burroughs Wellcome & Co. in 1 c.c. ampoules. The Sachs-Georgi test appears to be accepted as being reasonably accurate, and there is much to commend it in places where the Wassermann test cannot be carried out.

*The author wishes to express his gratitude to the Rev. J. K. Macgregor, the Rev. Father Ronayne and Mr. Hart, all of whom were most considerate in allowing him to examine the pupils attending the schools of which they are in charge. The author's thanks are also due to the Honourable the Director of the Medical Service, Nigeria, for permission to publish this article.

INCIDENCE OF YAWS IN CHILDHOOD

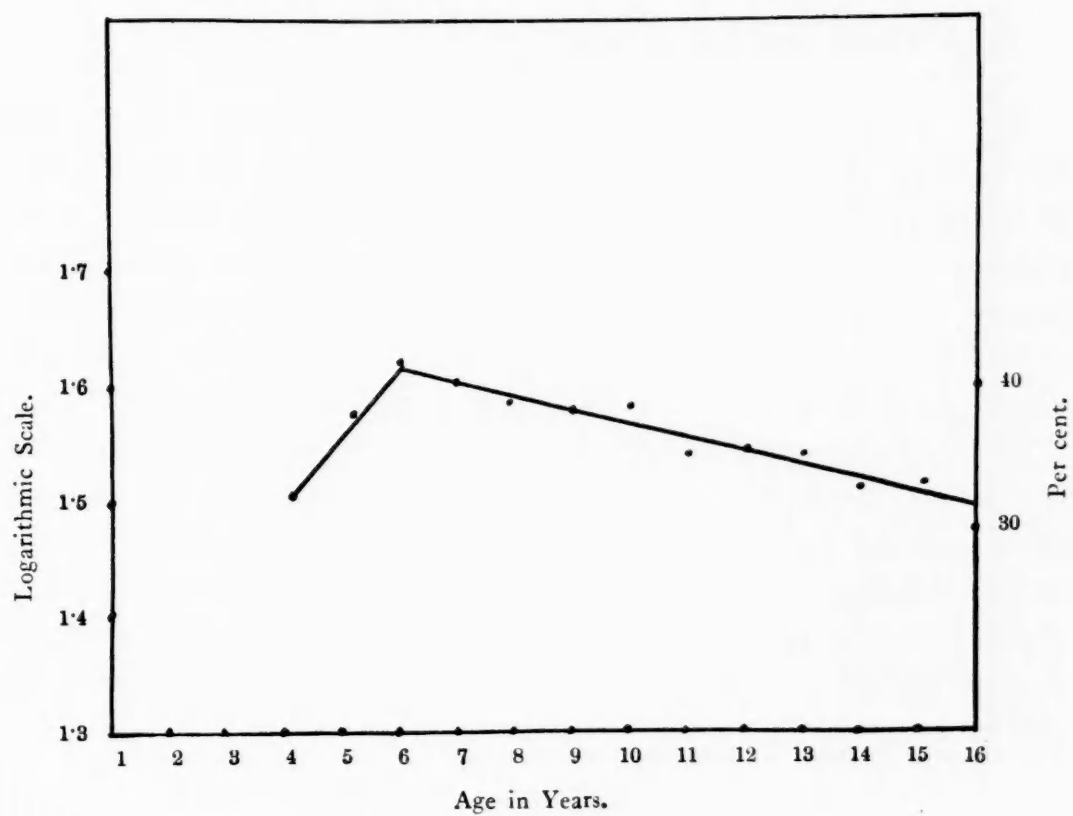
No serological test has yet been evolved by which yaws can be differentiated from syphilis ; and, therefore, in places where the two diseases co-exist, a positive reaction to a test such as the Wassermann or Sachs-Georgi indicates only that the individual has or has had either yaws, syphilis, or both. Clinically, the differential diagnosis is often difficult, and, in consequence, workers may vary in their assessment of the incidence of syphilis or yaws in the same locality. The difficult cases are chiefly those presenting the tertiary lesions seen in adult life, and even experienced clinicians will readily admit that in such cases it is frequently difficult, if not impossible, to distinguish yaws from syphilis. On the other hand, in childhood it is the characteristic primary and secondary lesions of yaws which most commonly occur, and the diagnosis is, therefore, rarely in doubt. Further, among children of sixteen years of age and under, acquired syphilis is most unlikely to occur, even in the tropics, and congenital syphilis is so rarely encountered (0.04 per cent. of patients in Calabar) that there is justification for eliminating syphilis as a factor in childhood ; and it seems reasonable, therefore, to assume that in children under sixteen years of age a positive Sachs-Georgi reaction is due only to infection with *Sp. pertenue*. If this assumption is accepted, then it follows that the figures given below indicate the average incidence of yaws in Calabar children, and that they present a more accurate indication of the extent of the disease than is possible by clinical examination alone, because a serological study will reveal the presence of yaws in cases where signs and symptoms remain latent or have passed off.

The striking fact which emerges from Table I is the high incidence of yaws in Calabar. Of two thousand six hundred children examined, the serum of no less than 35.6 per cent. gave a positive Sachs-Georgi reaction. These figures only bear out clinical observations which show that yaws is extremely prevalent in the Calabar area. Graph I, in which the results are plotted out on a logarithmic scale, shows that from the fourth year there is a rapid rise in the incidence of yaws, and that it reaches its maximum by the sixth year. Thereafter, until the sixteenth year, there is a steady fall in the proportion of infected children, a fall which averages about 1.3 per cent. per

TABLE I
Incidence of Yaws in early life

Age						Total	Positive Sera
							Percentage
4	200	32
5	200	39
6	200	42
7	200	40
8	200	38
9	200	37
10	200	37
11	200	34
12	200	35
13	200	34
14	200	32
15	200	32
16	200	31

GRAPH I. Incidence of Yaws in childhood, Calabar, Southern Nigeria.



annum. Between the ages of six and sixteen years the following simple formula seems to hold good :—

$$\text{Log } S^a = K - (A - 1) \text{ Log } d.$$

where S^a is the percentage of children with a positive Sachs-Georgi reaction at age A . In the present series, $K = 1.69$ and $\text{Log } d = 0.014$.

What is the explanation of this gradual decline in the incidence of yaws as adult life approaches? Surely the figures indicate that after the sixth year there is a tendency for the disease to burn itself out and for spontaneous cure to occur—a conclusion which is not entirely unsubstantiated, because Schöbl (1928) has observed the same phenomenon in his work on experimental yaws in monkeys. Further, these figures suggest that re-infection with yaws is not common during this period. It is very seldom that the serum of an untreated case of syphilis becomes negative spontaneously, and yet syphilis is caused by a spirochaete morphologically indistinguishable from *Sp. pertenue*. Without venturing to discuss the vexed question of whether or not yaws and syphilis are one and the same disease, this tendency to spontaneous cure, as manifested by the change from positive to negative in the serological reaction of a proportion of children with yaws, is quite a good argument in favour of the view that they are separate and distinct diseases.

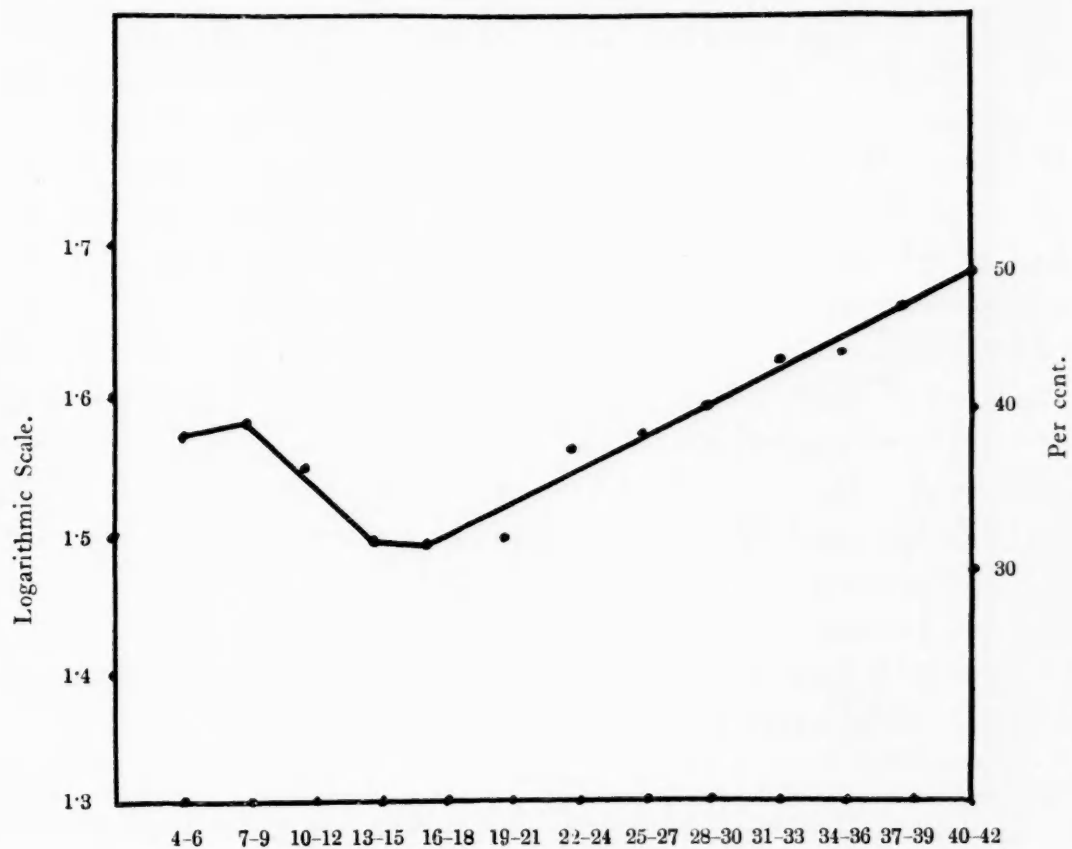
THE SACHS-GEORGI TEST IN ADULT LIFE

In our analysis of the results obtained with the Sachs-Georgi test in early life, it was suggested that a positive reaction is indicative of infection with yaws alone, because it is held that syphilis, either congenital or acquired, is a negligible factor at this period. Our results also seemed to show that re-infection with yaws is not common in childhood. Among adults, on the other hand, the whole problem of the interpretation of a positive serological reaction is much more complicated. A positive Sachs-Georgi reaction may be due to infection with syphilis, or yaws, or both; and, in consequence, the full onus of diagnosis rests with the clinician who, unfortunately, can expect little help from the laboratory. Table II shows that among adults, contrary to what takes place in childhood, the proportion of positive reactions steadily increases with the age of the subject.

TABLE II.
Sachs-Georgi Test in adult life.

Age group						Total	Positive Sera
							Percentage
16-18	452	32
19-21	223	32
22-24	261	37
25-27	418	38
28-30	431	40
31-33	250	43
34-36	283	44
37-39	145	47
40-42	137	50

GRAPH II. Sachs-Georgi reaction in childhood and adult life, Calabar, Southern Nigeria.



It has been shown that in childhood the proportion with positive sera falls at the rate of 1·3 per cent. per annum, whereas it will be seen from Graph II that in adult life it rises by exactly half this figure, viz. : 0·65 per cent. per annum.

Among adults from eighteen years of age onwards the results may be expressed by the formula :—

$$\text{Log } S^a = K + (A - 1) \text{ Log } d$$

and in this series $K = 1·365$ and $\text{Log } d = 0·00813$.

This rise in the proportion of positive sera as age advances is to be accounted for in two ways, each of which is a factor, viz. :—infection with syphilis, and infection and re-infection with yaws. Contrary to what obtains in syphilis, it is a well-established fact that individuals who have suffered from untreated yaws may become re-infected with the same disease, and, further, it has also been proved both clinically and experimentally (Schöbl, 1928) that primary and secondary yaws may develop in a patient who, at the same time, bears the stigmata of tertiary yaws, this phenomenon being known as super-infection.

Now, if the spontaneous 'burning out' of yaws which has been observed in childhood were to continue at a uniform rate throughout adult life, then by the age of forty to forty-two years only 6 per cent. would be serologically positive; and yet actually we find that yaws and syphilis together are present in about 50 per cent. at this age. It may be that the difference between the expected and the actual figures—44 per cent. in this instance—represents the combined incidence of syphilis and re-infection with yaws. On the other hand, we do not know for certain that the decline in the incidence of yaws observed in childhood does continue at the same uniform rate in adult life; and, in fact, it is just as probable that it falls to a certain figure and there remains constant. The only method by which this point could be settled would be to make a comprehensive serological survey of the people in an area where yaws is prevalent and where syphilis is unknown. The writer is not aware if such an investigation has been undertaken anywhere by a reliable authority.

From a serological study such as is described here it is impossible to offer any opinion as to the ratio of syphilis to yaws among adults in Calabar. Figures from the out-patient department and wards

of St. Margaret's Hospital, Calabar (1929), indicate that the ratio of syphilis to yaws is 45 to 55. These figures are based on observations of a large number of cases attending the hospital each year, but, while they are of considerable statistical value, they do not help us much in this study, because they are based on total attendances irrespective of the age of the subject. It is a recognised fact that, in Calabar, yaws is much more common during childhood than in later life, and, therefore, since a large number of children attend the hospital, it is quite possible, indeed almost certain, that among adults the ratio of syphilis to yaws is higher than the figures quoted above would indicate. What this ratio is remains unknown, and, unfortunately, serological tests are of little or no assistance in computing it. The only conclusion which is justified by our analysis is that, in Calabar, there is a steady decrease in the incidence of yaws between the ages of six and sixteen years ; and that thereafter, as age advances, there is a steady increase in the proportion giving a positive serological reaction—a reaction which may be due to syphilis, to yaws, or to re-infection with yaws. Further, there is reason to believe that the decline in the proportion of positive sera in childhood, and the rise observed in adult life, may be expressed by simple mathematical formulae.

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TREATMENT OF RHODESIAN SLEEPING SICKNESS WITH BAYER 205 AND TRYPARSAMIDE: OBSERVATIONS ON 719 CASES

BY

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(Received for publication 3 May, 1932)

INTRODUCTION

All the earlier literature on the therapeutic action of Bayer 205 and tryparsamide has been summarised by Yorke (1925). The only important series of Rhodesian cases *treated with Bayer 205 alone* mentioned in this review were thirty-five cases reported by Kleine and Fischer (1922, 1923, 1924). These, together with three others belonging to the same series, were subsequently reported on by Kinghorn (1926). Of these thirty-eight cases (twenty-one of whom were in a late stage of the disease at the commencement of treatment), thirty-five received a minimum quantity of 3·6 grammes of Bayer 205, usually within a period of twenty-eight days. Thirty of them died, one from sleeping sickness, five from intercurrent disease, and in twenty-four the cause of death was unknown. Of the remainder, one could not be traced, and four were alive and well nearly three years afterwards.

Of the three other cases who received less than 3·6 grammes, one was alive three years later. This last case (Chimuwira) is of interest in that he was reported by Kinghorn to have been ill for sixteen months before commencing specific treatment, which consisted of only two subcutaneous injections of Bayer 205, in 1·5 gramme doses, with an interval of three days between.

Dye (1926) reported twelve cases of whom ten remained well for periods of two to six months. The treatment was 6 to 7 grammes in 1 gramme doses over a period of twenty to thirty-eight days. From unpublished medical reports it appears that, eventually, ten in all of these cases died, either from sleeping sickness or inter-current disease.

Keevill (1926) reported twenty-six cases. The maximum total dosage of Bayer 205 was 5 grammes. During an observation period of three to four months, four died and one relapsed. This author (1928) recorded the subsequent history of six of these cases. These six are of special interest in being the first series published which show that sterilisation of the cerebro-spinal fluid can sometimes follow the administration of Bayer 205. One case received a total dosage of 3 grammes, and each of the other five a total of 5 grammes. Trypanosomes had been demonstrated in the cerebro-spinal fluid of all six at the beginning of treatment. More than two years later all of them were in excellent health, and in five, in whom it was examined, the cerebro-spinal fluid was found to be normal.

Kleine (1928) treated eight cases (Nos. 35, 51, 68, 70, 71, 73, 75, 76) at Ikoma in 1926-7, and these were subsequently reported on by Corson (1928, 1931). All of them except one (a child of seven years who received 2 grammes) received a minimum of 3 grammes, and most of them were given 5 to 7 grammes over periods of eight to thirty-nine days (except No. 35, where the period was one hundred and fifty days), the interval between the first and second doses never being more than four days. This series were given small quantities of other drugs which probably did not materially affect the treatment.

Three of the cases survive three or more years after the completion of treatment. The others succumbed in spite of the fact that most of them were re-treated after they relapsed. Two of the survivors were early or mild cases, while the third was ill three months before treatment. Of those who died, all but two were late cases.

Maclean (1929) reported twenty-two cases, only seventeen of whom received adequate treatment.

These seventeen (except in the case of children, who received

proportionately smaller doses) received a minimum of 3 grammes in a period of fifteen to thirty days. Only six were alive two to three years after, and one of these had relapsed. The five who were well (one of whom received less than 3 grammes) were all early cases. Three other early cases had died.

Very few Rhodesian cases *treated with tryparsamide alone* have been recorded. Dye (1926) treated twelve cases. His method was to give 3 grammes every five to seven days until a total of 30 grammes was given. He found that the drug failed to sterilise the peripheral blood. Keevill (1926) reported eight cases. He gave three gramme doses weekly, the maximum number of injections being five. Only one patient showed improvement and he suffered from headaches. Maclean (1929) reported three cases. One case who received 24 grammes in a period of eight weeks was well more than two years later.

Combined treatment with Bayer 205 and tryparsamide has been employed by most workers in Tanganyika Territory since 1925. Keevill (1926) reported seven cases, four of whom were well after an observation period of six or more months. Corson (1928) recorded an early infection in a European who made a complete recovery after 8.3 grammes Bayer 205 and 22.9 grammes tryparsamide.

The same author (1930-1931) recorded nine late or relapsed cases. He found both Bayer 205 and tryparsamide unsatisfactory in the late stages. In one case his method was to treat the patient with tryparsamide on every occasion on which parasites appeared in the blood. In this way 11 grammes were given over a period of thirty days, only one injection being given when the blood was negative. This treatment did not completely sterilise the blood.

The results of treatment with Bayer 205 and tryparsamide both separately and combined, are reported in the annual medical reports of Tanganyika Territory from 1925 onwards. Except for a few that could not be traced, nearly all the cases previously recorded, as well as a number of new ones, are incorporated in the report for 1930, in which the general results in two thousand six hundred and ten cases after an observation period of one to five years are recorded.

In the series described in the present paper, most of the cases occurred in Tabora Province during the first and second years of an

epidemic which swept the Northern part of this Province from the end of 1927 to 1930. The other cases occurred between 1925 and 1927 in a part of Mwanza Province where sleeping sickness was epidemic in 1920-22 and endemic in subsequent years.

There were eight hundred and seventy-three cases in the original series. The records of ninety-one of these had to be discarded because the patients died before they completed their treatment, and for various other reasons. Besides these, there were sixty-three cases who could not be traced in 1931. The survivors of the remaining seven hundred and nineteen cases have all had their general clinical condition and also their blood and cerebro-spinal fluid examined from time to time since completing treatment. Altogether they have been kept under observation for a minimum period of two years and five months from the time that they commenced treatment, and some of the Mwanza ones have been observed for more than six and a half years.

GENERAL RESULTS OF TREATMENT

In the Mwanza (endemic) batch, consisting of ninety-one males and forty females, the routine treatment was 1 gramme of Bayer 205 on the first, tenth and twenty-eighth day, followed, in some of the cases, by about eight injections of tryparsamide, usually in 3 gramme weekly doses. Most of them were at an advanced stage when treatment commenced. The number still alive is twenty-seven (20.6 per cent.). Among these twenty-seven there were six (22.2 per cent.) relapsed cases who had to be re-treated.

In the Tabora (epidemic) batch, consisting of three hundred and sixty-six males and two hundred and twenty-two females, the routine treatment was 1 gramme Bayer 205 weekly for four weeks, followed in all the late cases, and in some of the early ones as well, by 2 to 4 gramme (usually 3 gramme) doses of tryparsamide, weekly, for twelve weeks. The interval between the last injection of Bayer 205 and the first injection of tryparsamide was seven days to one month. One small series received Bayer 205 at the first injection, tryparsamide at the second to fourth injections, Bayer 205 again at the fifth to eighth injections, and finally tryparsamide at the ninth to seventeenth injections. A third series, seventy in number, received

tryparsamide, twelve injections, in 2 to 4 gramme doses as a routine treatment.

The majority of the Tabora batch were relatively early cases. The number still alive is three hundred and eighty-two (64.9 per cent.). Of these survivors, ninety-nine (25.9 per cent.) relapsed and were re-treated. Twenty-five of the relapsed cases are still under treatment, more than two and a half years after the initial treatment.

In a large proportion of the cases who received combined Bayer 205 and tryparsamide treatment, the prescribed routine was not followed, the patients finding the course too long and irksome to attend regularly. The administration of Bayer 205 on the other hand, either when given alone or as the first part of the treatment, was generally carried out at the prescribed intervals, though the prescribed number of injections was not always given.

All ages were treated, from children a few months old to persons over fifty years. Table I shows the age distribution in the eight hundred and seventy-three cases.

In none of the cases, either adult or children, was the dose determined by weighing the patient.

In the majority of cases the drugs were given intravenously, but sometimes subcutaneously and intramuscularly.

The general survival rate after treatment is shown in Table II. It may be explained here that 'recovery' and 'survival' as used in the paper do not necessarily imply permanent cure, but simply refer to the apparent condition of the patient when last observed.

ANALYSIS OF CASES AND TREATMENT

The cases are divided into three categories, viz. :—

- CATEGORY I. Cases, the duration of whose illness was one month or less before treatment.
- CATEGORY II. Cases, the duration of whose illness was more than one month before treatment.
- CATEGORY III. Cases, the duration of whose illness before treatment is unknown, and also cases who complained of no symptoms and who were only discovered accidentally.

TREATMENT WITH BAYER 205

Table III shows cases belonging to Category I treated with Bayer 205. It includes all cases who are well without further treatment, and all deaths whether these had further treatment with other drugs after relapsing or not. It does not include relapses who had to be re-treated and who are still alive. Also it does not include three cases that appeared to be definite re-infections.

With the exception of Class 4 (i.e. cases receiving 4 grammes Bayer 205 in 21 to 24 days, viz. :—1 gramme weekly in most cases), the numbers are too small for comparison, but so far as they go they suggest that a total dosage of less than 4 grammes is inadequate in the average case.

Discarding Classes 1 to 3a, in which less than 3 grammes was given, the remaining one hundred and eight cases show eighty-nine recoveries—a rate of 82·4 per cent. The eight cases in Class 4a received 4 grammes during a period exceeding twenty-four days, and, if they are excluded, the recovery rate with regular Bayer 205 treatment in this category rises to 85 per cent.

Thirteen cases, still alive, who received a minimum of 4 grammes of Bayer 205, relapsed and were re-treated. If these are added to the total treated (in Classes 4, 4a and 5) and regarded as failures, the recovery rate is 73·5 per cent. This last figure may therefore be taken as the minimum recovery rate in this particular series.

In Category II, one hundred and two cases received Bayer 205 only as initial treatment. Eighteen of these received 2 grammes or less of the drug, and only two of the eighteen are now alive.

The remaining cases received from 3 to 5 grammes, and the results obtained in seventy-four of them are shown in Table IV. Relapsed cases who were re-treated and are still alive, ten in number, are excluded as in Table III. Class 3a in Table IV consists almost entirely of the Maswa cases whose routine treatment has already been described. (In seven cases in this class, six of whom died, the period during which treatment was given exceeded the twenty-eight days.) The results in Class 3a are distinctly poor. The numbers in Class 4 are too small for accurate comparison with the same class in Table III, but the difference is enough to demonstrate the importance of the time element before treatment.

In Category III, there are only fifteen cases who received Bayer 205 only, and two of these received less than 3 grammes. These two are dead. The remainder are included in Table V.

TREATMENT WITH TRYPARSAMIDE

Forty-one cases are recorded in Table VI. It is difficult to assess the value of this drug. It is more apt to show toxic symptoms in therapeutic doses than Bayer 205 and is also slower in its action. A number of cases, though clinically improved, show parasites in their blood or cerebro-spinal fluid after as much as 36 grammes administered over a period of three months.

Naturally, where progress was unsatisfactory, Bayer 205 was generally given. The result has been that the cases who received tryparsamide treatment only are, on the whole, a selected group, most of those who responded tardily to this drug being subsequently given combined treatment.

On the other hand, there are probably a number of cases who were given Bayer 205 before completing a tryparsamide course, but who might have responded in time to prolonged treatment with the latter drug.

It has not been possible, from a study of our protocols, to form an opinion as to the type of case that will respond to tryparsamide. Most of the cases recorded in Table VI commenced treatment within about two months of the onset of symptoms, and the most that can be said at present is that tryparsamide can apparently cure a considerable proportion of moderately early Rhodesian cases, and that, provided they are not too acute or advanced, or too susceptible to the drug, the proportion of recoveries (regarding relapses as failures) may sometimes be as high as 26 per cent.

COMBINED TREATMENT WITH BAYER 205 AND TRYPARSAMIDE

Excluding seventy-three who relapsed and were re-treated, one hundred and thirty-six cases in Category I, one hundred and fifty-four in Category II and twenty-two in Category III received the combined treatment. The general results are shown in Table VII. Many of the cases in Class 6 had their course of treatment cut short by death.

Cases belonging to Category I in Classes 10 and 11 of Table VII show a lower percentage of recoveries than Classes 4 and 5 in Table III (72.3 per cent. as against 85 per cent.). The real reason for this is the fact that many of the former cases, though in the same category as those in Table III, were in a more advanced stage and for this reason had been given fuller treatment.

A comparison between the cases belonging to Category II in Table VII with those in Table IV shows some apparent advantage in favour of combined treatment.

In Table IV, Classes 4, 4a and 5 give a recovery rate of 27.2 per cent. in forty-four cases, or, if Class 4a, in which the treatment was not so regular, is excluded, 28.1 per cent. in thirty-two cases. These figures, as they stand, cannot legitimately be compared with those belonging to the same Category in Table VII, since examination of the protocols shows that ten of the twenty-two deaths in Class 4, and one in Class 4a in Table IV, all occurred within a month of the last injection of Bayer 205; that is, they did not live long enough to get a course of tryparsamide had it been prescribed for them.

If these eleven cases are excluded, the recovery rate in Classes 4, 4a and 5 combined becomes 36.3 per cent. in thirty-three cases, or in Classes 4 and 5 combined, 40.9 per cent. in twenty-two cases.

In Table VII, Classes 9, 10 and 11 combined give a recovery rate of 46.9 per cent. in ninety-eight cases, and Class 11 alone 52 per cent. in forty-eight cases.

It has to be noted that this last class includes cases who received their treatment very irregularly, whereas those in Class 4 in Table IV received their treatment regularly and within the prescribed period.

The better results shown in Class 11, with its higher tryparsamide dosage, as compared with 10 are not due to the inclusion in the former of cases who received 6 grammes of Bayer 205. There were four such cases, but only one of them survived.

A curious point is revealed in this table in connection with Class 7. The recovery rate for all categories combined in this class is 63.4 per cent. in fifty-two cases, a higher rate than that of any other class, except 11, where the rate is 63.6 per cent. in eighty-eight cases. In Category II alone the results are not so striking, but the rate is higher in Class 7 than in either Class 8 or 9.

This apparent good result may be a fallacy arising out of individual

variations in the small numbers observed ; on the other hand, it may be due to the fact that, after the preliminary administration of one or two injections of Bayer 205, the cases received early tryparsamide treatment.

If this were so it would explain the better results afterwards to be seen in Table IX.

Table VIII shows a small series extracted from Table VII, in which the treatment was regular and consisted of four injections of Bayer 205 in 1 gramme weekly doses, followed, after an interval of from one week to about one month, by twelve injections of tryparsamide in 2 to 4 (usually 3) gramme weekly doses. This series gives a recovery rate of 56.2 per cent. in sixteen cases belonging to Category II.

In the series in Table IX, also extracted from Table VII, the routine treatment was:—one injection of Bayer 205, four injections of tryparsamide, four injections of Bayer 205, eight injections of tryparsamide. The interval between the injections was one week, irrespective of the drug used. The dose of Bayer 205 was 1 gramme, and of tryparsamide 2 to 4 (usually 3) grammes.

Regarding two cases who are alive after re-treatment as failures the cases belonging to Category II in this table show a recovery rate of 59 per cent. in twenty-two cases.

Another comparison between Bayer 205 and combined treatment is made in Table X. In this table are included cases that relapsed (including possible re-infections) after the prescribed course of treatment.

It may be assumed that the relapsed cases would have died had they received no further treatment.

The numbers obtained from these tables are too small to form reliable guides, but they do suggest that tryparsamide, properly administered, increases considerably the recovery rate in advanced cases. Table VII in particular suggests that in combined treatment the total dosage of Bayer 205 in adults should not be less than 4 grammes and that of tryparsamide not less than 22 grammes.

The value of combined treatment is perhaps best shown in cases whom Bayer 205 failed to sterilise and who subsequently recovered after a course of tryparsamide. Examples of such cases are shown in Table XI. It may be objected that in these

cases the action of Bayer 205 was delayed and that recovery would have taken place without further treatment. We have no record of such a recovery in any of our cases, but Van den Branden (1926) reports two Gambiense cases who had blood relapses after Bayer 205 and subsequently appeared to have recovered without further treatment.

In the combined treatment series, the general method followed was that described for the cases in Table VIII, modified in many instances by the irregular attendance of the patients. Besides this method and that described in Table IX, a number of cases who received tryparsamide to begin with were given Bayer 205 afterwards, either as a precaution or because progress was not satisfactory. This last method has distinct disadvantages in acute and advanced cases on account of the slow response to tryparsamide, and, though in certain cases the method appears to act as effectively in the end as any of the others, we have not found any type of case in which it has special advantages.

In the 1928 Annual Medical Report for the Territory, there is a record of some cases who came for treatment when the disease was at such an advanced stage that the patients could not attend to their own wants.

None of those who received Bayer 205 treatment recovered, whereas a few did so who received combined treatment with this drug and tryparsamide.

THE RELATIONSHIP OF THE DURATION OF THE ILLNESS TO DIFFERENT SIGNS

One hundred and thirty-two cases recorded in Table XII show the correlation between the presence of oedema, the number of cells in the cerebro-spinal fluid and the duration of illness before treatment. It will be observed that with few exceptions every case showing oedema had a definite meningeal reaction. The exceptions were one case of leprosy, one in whom the oedema appeared to be due to other causes and a few apparently uncomplicated cases who showed just a trace. On the other hand, there may be well-marked meningeal involvement with absence of oedema.

It is difficult to correlate the cell counts with the duration of illness. We are dependent on the statements of patients and their

friends for histories, but even after allowing for discrepancies that may have arisen in this way, it does not appear to be possible to judge with any accuracy the duration of the disease from cytological examination of the cerebro-spinal fluid, at least when the number of cells exceeds 10 per c.mm. This is partly due to the wide margin of error that must exist in cell counts.

Findings which show normal cell counts on the one hand or the presence of parasites on the other are, of course, of immense value. As will be seen later, examination of the cerebro-spinal fluid is most important in the study of relapses.

FALLACIES IN THE CYTOLOGICAL EXAMINATION OF THE CERE BRO-SPINAL FLUID

Certain forms of syphilis have, of course, to be excluded. It is possible that, in some of our cases, this disease was present, unsuspected, and that the administration of tryparsamide produced a cytological change analogous to what it produces in late trypanosomiasis. Unless something like this has occurred, syphilis has not been an important source of fallacy in our experience.

Serious fallacies can arise from intercurrent infection with relapsing fever, a disease which, in Africans, we have frequently found to respond well to Bayer 205. The following cases illustrate this :—

A youth of about fifteen years of age, suffering from fever and a slight retraction of the neck, was found to have numerous spirochaetes (*S. duttoni*) in his blood. The cerebro-spinal cell content was 1080 per c.mm.

One gramme of Bayer 205 was given, and his temperature became normal in twenty-four hours. A week later the cerebro-spinal cell count was normal.

A case of sleeping sickness (diagnosed microscopically) of moderate severity was found to have a cerebro-spinal cell content of 1100 per c.mm., and, near the time of the examination, he was given one gramme Bayer 205.

Two days after the first examination he was lumbar-punctured again, and some of the fluid, whose cell content was now down to 280 per c.mm., was inoculated into a monkey. This animal

subsequently showed spirochaetal infection. A natural infection of the monkey, though unlikely, cannot definitely be excluded.

Sometimes, in mixed infections, the spirochaete can be demonstrated by inoculation of the infected blood into rats when it is not found in films.

THE ACTION OF BAYER 205 ON THE CEREBRO-SPINAL FLUID

Of thirty-nine cases with trypanosomes in the cerebro-spinal fluid at the commencement of treatment (usually examined the day after the first injection), who received 4 grammes of Bayer 205 over a period of twenty-one to twenty-nine days, thirteen recovered without further treatment, thirteen others are alive, but relapsed and were re-treated, and thirteen are dead. Twenty-one of these cases are shown in Table XIII.

ACTION OF BAYER 205 FOLLOWED BY TRYPARSAMIDE ON THE CEREBRO-SPINAL FLUID

Of thirty-two cases with trypanosomes in the cerebro-spinal fluid at the beginning of treatment, who received at least 3 (usually 4 or more) grammes Bayer 205, and at least 21 (usually 30 or more) grammes tryparsamide, twenty remained well without further treatment, seven others are alive but relapsed and were re-treated, and five died.

The cases that recovered after tryparsamide treatment alone did not have their cerebro-spinal fluid examined at the commencement of treatment.

PROGNOSIS

So far as our observations go, uncomplicated cases, treated with adequate doses of Bayer 205 within the first few days of onset of symptoms, or before there is a meningeal reaction (anything over 3 cells per c.mm. may be regarded as a meningeal reaction), invariably recover.

After the first week or after a meningeal reaction has set in, the prognosis, though very good for about a month or so, is no longer certain. Conversely, only the most advanced cases need be regarded as hopeless, as surprising recoveries occasionally take place.

Owing to the fact that the great majority of our cases are periodically exposed to re-infection, it is difficult to collect exact data bearing on prognosis. Lumbar puncture, for instance, which might serve as a valuable guide, can rarely be performed oftener than every three months in treated cases, and usually it can only be done once in six months or a year. Even the shorter interval of three months is sufficient to allow re-infection of the cerebro-spinal fluid and an altered cytological picture to take place.

The following cases illustrate the difficulty :—

CASE 588.

Treated with 4 grammes Bayer 205 in a period of 22 days.

Cerebro-spinal fluid :

At the beginning of treatment, 10 cells per c.mm. No parasites.

At the end of treatment, 3 cells per c.mm. No parasites.

Two months after commencement of treatment, 3 cells per c.mm. No parasites.

Eleven months after commencement of treatment, 163 cells per c.mm. No parasites.

Case re-treated but subsequently died.

CASE 626.

Treated with 4 grammes of Bayer 205 in a period of 22 days and 18 months, afterwards with 6 grammes of tryparsamide in the course of a month.

Cerebro-spinal fluid :

At the beginning of treatment, 30 cells per c.mm. Trypanosomes present.

At the end of Bayer treatment, 210 cells per c.mm. No trypanosomes.

At the beginning of tryparsamide treatment (i.e., 19 months after commencement of Bayer 205), 80 cells per c.mm. No trypanosomes.

At the end of tryparsamide treatment, 0 cells per c.mm. No trypanosomes.

Four months later, 436 cells per c.mm. Trypanosomes present.

Patient subsequently died.

CASE 749.

Treated with 4 grammes Bayer 205 in a period of 22 days, and, after an interval of one month, with 36 grammes tryparsamide in a period of 78 days.

Condition of the cerebro-spinal fluid :

At the beginning of treatment, 63 cells per c.mm. No parasites.

One month after treatment, 33 cells per c.mm. No parasites.

Four and a half months after (i.e., just after completion of tryparsamide), 0 cells per c.mm. No parasites.

Eight and a half months after, 0 cells per c.mm. No parasites.

Seventeen months after (i.e., 8½ months after last examination), 78 cells per c.mm. No parasites.

Re-treatment commenced.

Patient was well 35 months after commencement of original treatment.

CASE 92.

Treated with 2 grammes Bayer 205 and 6 grammes tryparsamide in 9 days.

Condition of the cerebro-spinal fluid :

At the beginning of treatment, 40 cells per c.mm. Trypanosomes present.

Four and a half months after, 0 cells per c.mm. No parasites.

Eight months after (i.e., $3\frac{1}{2}$ months after last examination), 273 cells per c.mm. Trypanosomes present.

Re-treated with 36 grammes tryparsamide. Well, with only one cell per c.mm., three years after commencement of treatment.

In none of these cases, except possibly No. 749, can re-infection be excluded.

Generally, when a rise in the cell content of the cerebro-spinal fluid has been observed after treatment, it was assumed that a relapse was impending, and, as the interests of the patient had to be the prime consideration, additional treatment was usually given without waiting to see what course the case would otherwise take. Many such cases got well, and it is not possible to say definitely how far the rising cell content would have been a bad prognostic sign had no more treatment been given.

We only know of one case (US29) with a cell count persistently over 15 per c.mm. for a year who remained well without re-treatment, and this case was not under continuous observation. He had 26 cells per c.mm. in September, 1929, and 30 per c.mm. in November, 1931. The only time that he was seen during the interval was in September, 1931, and he was well then.

Cases with a cell content over 200 per c.mm. at the beginning of treatment may recover. In nineteen such cases, seven are well without re-treatment, and two more after re-treatment.

Early or moderately early cases, who are going to recover, present a fairly constant picture as the following cases show (p. 171).

The last case (58) illustrates recovery after a long delay in the fall of the cell content, the difference between the first and second readings in time being five months.

Failure to find trypanosomes in the cerebro-spinal fluid has some prognostic significance in masses of cases, provided a uniform technique is employed. That employed in all our later cases was to examine two successive drops of fresh cerebro-spinal fluid in the chamber of a Thoma-Zeiss or Turck haemocytometer before declaring a specimen negative.

In sixty-two cases whose cerebro-spinal fluids were negative but with a cell content of over 5 c.mm., the recovery rate was 77.4 per cent., while in thirty-four cases with positive cerebro-spinal fluids the recovery rate was 47 per cent. (See Table XII for particulars.) This difference is partly due to the fact that, as a rule, after invasion of the cerebro-spinal fluid, the parasites are more scanty in the early stages of the disease.

Condition of the cerebro-spinal fluid.

Case		At beginning of treatment	7-8 days after	21-28 days after	1-6 months after	6-12 months after	1-2 years after	Over 2 years after
413	Cells per c.mm. ...	15	60	0	...	0	...	6
	Trypanosomes ...	Neg.	Neg.	Neg.	...	Neg.	...	Neg.
702	Cells per c.mm. ...	17	17	0	2	2
	Trypanosomes ...	Neg.	Neg.	Neg.	Neg.	Neg.
789	Cells per c.mm. ...	240	10	27	3-0	2
	Trypanosomes ...	Neg.	Neg.	Neg.	Neg.	Neg.
734	Cells per c.mm. ...	3	13	13	0	7
	Trypanosomes ...	Pos.	Neg.	Neg.	Neg.	Neg.
622	Cells per c.mm. ...	3	17	15	7	1-3	...	4
	Trypanosomes ...	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
106	Cells per c.mm. ...	30	5	1-3
	Trypanosomes ...	Pos.	Neg.	Neg.
58	Cells per c.mm. ...	45	40	4
	Trypanosomes ...	Neg.	Neg.	Neg.

The protein content of the cerebro-spinal fluid was only done in some relapsed cases of this series. So far as our observations have gone the results are generally analogous to those obtained with cytological examination.

Among more than two hundred cases whose cerebro-spinal fluid was examined at the beginning of treatment, there were nine who showed no cells in volumes of $\frac{3}{10}$ th c.mm. of the fluid. All except one of these recovered, and the cause of death in this case was unknown.

The general condition of the patients after treatment is, as a rule, no guide to prognosis. Some patients who appear to be in excellent physical condition and do hard work for months, may afterwards relapse. Frequently, if the cerebro-spinal fluid of such cases is examined, the cell count is high and it may show parasites before the patient complains of any symptoms.

In the present stage of our knowledge the prognosis must be based almost entirely on the state of the patient at the *beginning* of treatment, particularly with regard to his general health, the duration of the illness and the state of the cerebro-spinal fluid.

THE INFLUENCE OF AGE, SEX AND INTERCURRENT DISEASE

The commonest intercurrent disease met with was ankylostomiasis.

All the cases in the Tabora batch were not examined for ankylostomiasis at the beginning, but subsequent examination, both of trypanosomiasis cases and of the general population, makes it probable that practically all of them had this infection. In Ikoma, on the other hand, Corson (1928) found no ankylostome infection among his cases in the course of a whole year.

Kleine's (1928) and Corson's (1928-1931) Ikoma cases received, on an average, fuller and more intensive Bayer 205 treatment than the Tabora cases. The general result (Corson, 1931) was 45.3 per cent. recoveries in sixty-four cases, and the best result, 63 per cent. in twenty-seven cases; while in the Tabora group the general result was 64.9 per cent. in five hundred and eighty-eight cases (including as failures cases who were inadequately treated), and the best result at least 73.5 per cent. in one hundred and twenty-one cases.

It is unlikely therefore that moderate ankylostome infection has any important influence on the results of treatment.

The incidence of age and sex and their survival rates are shown

in Table II. The survival rate among males and females is shown to be approximately the same. The survival rate among the different age groups varies somewhat, but, in the groups where the variation is greatest, the actual numbers are so small that the apparent variation has probably no significance.

It should be stated, however, that it is now found that children can tolerate both drugs relatively well, and that much larger doses could have been given than the cases in the 0-5 years group received. It is possible, therefore, that the lower recovery rate in this group may have been due to inadequate dosage.

RELAPSES

Under relapses are included cases who, after a temporary improvement, showed a return of symptoms, usually with parasites, either in the blood or cerebro-spinal fluid or both; cases whose blood or cerebro-spinal fluid was not sterilised after a course of treatment, though showing clinical improvement; and cases that are not clearly re-infections.

Among the cases recorded in this paper who were over fifteen years of age, fifty belonging to Category I and eighty-three belonging to Category II relapsed and were re-treated. Among the former, 38, or 76 per cent., and among the latter 36, or 43.3 per cent., remain alive. This disparity appears to be due to the fact that cases in Category I who received inadequate treatment react better, after relapse, to a full course of treatment than cases who were more advanced. It may be observed from Table X that those belonging to Category I who have relapsed after a full course react to re-treatment only a little better than those of Category II, viz :—fourteen out of twenty-three cases in the former, and eight out of sixteen in the latter.

How far such relapses can be prevented by prolonged treatment is still uncertain. Dr. F. V. Adams in this Territory commenced, in 1930, the treatment of a series with prolonged administration of Bayer 205. This series is still too small and has been under observation for too short a time to warrant any conclusions, but six of his cases may be mentioned to show that prolonged and intensive treatment is not always successful.

These six cases had been ill two to four months before beginning treatment, and were all still in the ambulant stage. The treatment prescribed was as follows :—

1 gramme Bayer 205 on 1st, 4th, 7th, 14th and 21st days.

Interval 3 weeks.

4 grammes Bayer 205 in 1 gramme doses over a period of 14 to 21 days. Interval 1 to 3 weeks.

4 grammes Bayer 205 in 1 gramme doses over a period of 14 to 21 days. Interval 1 to 3 weeks.

5 grammes Bayer 205 over a period of 21 to 29 days.

The routine was followed approximately by all except one case, who showed parasites in his cerebro-spinal fluid after his ninth injection and was afterwards given tryparsamide.

Two of the remaining five had true relapses within thirteen months of receiving their fourteenth injection of Bayer 205; and one remained in such indifferent health with a high cell content of his cerebro-spinal fluid that he had to be given more treatment.

The duration of the quiescent period between the completion of the treatment and the onset of relapse is very variable, and its greatest length is difficult to determine. Some have come back with parasites in the peripheral circulation as late as thirty-two to thirty-six months after completion of treatment, but as those cases had not been under observation for thirty-one or more months before, they could quite well be re-infections, irrespective of what was found in the cerebro-spinal fluid. Of cases who, because of regular observations, can be regarded as true relapses, the longest quiescent period was ten months, and three to six months the commonest. It is thus impossible to say at this stage how many months, or even years, a late case has to be kept under observation before he can be pronounced cured.

The treatment of the relapses in most of the cases has been on the same lines as that for ordinary late cases. Various modifications of prolonged and intensive treatment are being tried. The results are disappointing in most cases, but it is too early yet to draw any conclusions.

RE-INFECTIONS

Reference to some points that may serve to distinguish certain cases of re-infections from true relapses was made in the Annual Report for the Territory for 1930.

A true relapse usually, and probably always, shows a high cell content of his cerebro-spinal fluid, and if he has been neglected he generally gives a history of indifferent health extending sometimes over months before he becomes incapacitated.

Occasionally a type of case is met with where the patient, after a period of good health following upon specific treatment, develops fever with trypanosomes in the blood but a cerebro-spinal cell content which may be as low as 3 per c.mm. A case of this type is in all probability a re-infection.

In cases that appeared to be re-infections (including those whose cerebro-spinal fluid was not examined at the time of re-appearance of trypanosomes in the blood), the shortest interval between the completion of treatment and the re-discovering of the parasites was seventeen months. Only a few have been observed and it is quite possible that shorter intervals may yet be met with.

In these apparent re-infections the immediate response to re-treatment is good.

TOXIC EFFECTS OF BAYER 205

With this drug albuminuria, usually transitory, was not infrequently met with.

It was not practicable, with the staff at our disposal, to do repeated examinations of the urine in every case, but, so far as our observations go, albuminuria is not an important factor in cases receiving 1 gramme weekly doses of the drug. The condition frequently clears up without discontinuing treatment.

In some relapsed cases the condition may be found to begin after re-treatment and to persist till death, particularly if large doses (1.5 to 2 grammes) are given.

A dermatitis was met with in a few cases. A description of this condition has already been published (Maclean, 1928).

The first two cases observed with this condition died. One observed by Dr. D. E. Wilson in 1929 recovered.

TOXIC EFFECTS OF TRYPARSAMIDE

Visual disturbance occurred in thirty-nine of the cases under review, and fourteen of them became completely blind. Many more cases have been met with in the course of our work. The amount of tryparsamide that different individuals, and also the same individual under different conditions, can tolerate varies considerably, and is not entirely dependent on body weight.

Many cases, particularly early ones, can tolerate as much as 46 to 48 grammes in a period of seventy-eight days. Others show visual disturbance after 15 grammes or less, given in 3 gramme weekly doses. One case, a young man of twenty-five years, who had been acutely ill for less than a week, became blind after two injections of 3 grammes each, given at an interval of a week.

Except in relapsed cases we have not observed blindness to follow 2 gramme weekly doses, even after eight injections.

Relapsed cases appear to be much more susceptible, possibly because of tissue damage due to disease, possibly because of previous administration of tryparsamide. The following case is an example :—

CASE. Namwiza.

Treated with 1 gramme Bayer 205 and 12 grammes tryparsamide.

There was no visual disturbance observed.

Relapsed 32 months later and treated with 1 gramme Bayer 205 and 12 grammes tryparsamide in 2 gramme weekly doses. There was dimness of vision after the last injection.

Eleven months later the patient was ill again and six injections of Bayer 205 in 1.5 gramme weekly doses were given, followed by one injection of 2 grammes tryparsamide. There was transitory albuminuria during the Bayer 205 administration which cleared up before the course with this drug was completed. Within a week of the administration of the single dose of tryparsamide, dimness of vision had set in.

The onset of blindness is sometimes gradual, sometimes quite sudden. It may not appear immediately after the administration of the drug. For instance, a patient may leave hospital without any complaints after completion of his treatment, and become blind some time after going home. As this has occurred in patients after they left off being under continuous observation, we are unable to say what the length of this 'latent period' may be.

Dimness of vision without complete blindness frequently clears up. In our cases it always occurred in patients where visual acuity before

treatment was not even approximately known. They were Africans whose everyday work did not require keen eyesight and who might easily fail to detect a slight impairment of vision in themselves. It is difficult to say in such cases whether visual acuity is completely restored or not.

One case has gross impairment without blindness for a period of eighteen months, and is showing no signs either of improving or getting worse.

Once complete blindness sets in, we have never known visual recovery to take place.

The prevention of blindness is practically impossible in a proportion of relapsed cases whose prognosis is grave, if they are to receive effective treatment with tryparsamide.

SUMMARY AND CONCLUSIONS

The treatment of seven hundred and nineteen cases is analysed, and it is shown that with adequate administration of Bayer 205 (4.5 grammes in 1 gramme weekly doses) as many as 73.5 per cent. may recover and remain well for a period of twenty-nine or more months, provided treatment is begun within a month of the onset of symptoms. This drug is effective in the early stages of infection of the cerebro-spinal fluid. Generally speaking the earlier the case the better the chance of cure, and if an uncomplicated case is treated at the onset of symptoms complete recovery is practically certain.

It is also shown that a number of cases recover after administration of tryparsamide alone, but it is much less rapid, less effective and less constant in its results than the former drug.

There is no conclusive proof, from a study of these cases, that combined treatment with Bayer 205 and tryparsamide gives better results in moderately advanced cases than the former drug alone, but what evidence there is, supports the view that the combination is an advantage in late cases.

At a time when cases came for treatment at a more advanced stage of the disease than they generally do now, it was found that none of the cachetic cases ever recovered after the administration of Bayer 205 alone, whereas a few did so after the combined treatment.

For these reasons, combined treatment should always be given

when the prognosis is in doubt, particularly as the records suggest that there are some strains or phases which respond specially well to tryparsamide.

The dose of tryparsamide should be 2-4 grammes in adults and a total dosage should be at least 22 grammes.

Except in relapses, where the results are not comparable with those obtained in previously untreated cases, we have a very limited experience of more intensive or prolonged administration of Bayer 205 than that described above.

It is possible that more intensive treatment would give better results in cases in whom the prognosis is doubtful.

Neither age, sex nor a common intercurrent disease like ankylostomiasis has been found to have any material influence on the results of treatment.

The toxic effects of Bayer 205, when administered as described, are rarely of practical importance.

The toxic effects of tryparsamide are more serious and cannot always be guarded against. In relapsed cases, who appear to be more susceptible, the initial dose should not exceed 2 grammes.

Prognosis is best arrived at by observing the condition of the patient just before treatment, the chief points to be noted being the duration of the illness, the state of the general health and the condition of the cerebro-spinal fluid. Examination of the patient a few weeks or months after termination of treatment may give quite deceptive results.

ACKNOWLEDGMENTS

We have received considerable assistance from various members of the Department, Medical Officers, Sub-Assistant Surgeons and subordinate staff in carrying out treatment and keeping records.

We are particularly indebted to Mr. Tote, Sub-Assistant Surgeon, for following up and making observations on a large number of the cases.

We have to thank the Honourable the Director of Medical and Sanitary Services for permission to publish this paper.

TABLE I
Age distribution of 873 cases
(See page 160)

Age group	0-5 years	6-15 years	16-25 years	26-50 years	Over 50 years	Age not recorded	Total
Number of males ...	11	51	122	329	3	31	547
Number of females ...	5	29	82	195	4	11	326
Total ...	16	80	204	524	7	42	873

TABLE II
Showing the survival rate among the 719 treated cases of the two sexes in the different age groups.
(See pages 161 and 173)

Age group	0-5 years	6-15 years	16-25 years	26-50 years	Over 50 years	Age unknown	Total	Percentage alive
Alive { M.	3	26	62	154	11	2	258	56.4
{ F.	0	15	47	85	3	1	151	57.6
Dead { M.	4	18	38	121	15	3	199	...
{ F.	1	14	24	65	5	2	111	...
Total	8	73	171	425	34	8	719	56.8
Survival Rate ...	37.5%	56.1%	63.7%	56.2%	41.1%	37.5%		

TABLE III

Showing cases belonging to Category I, who received Bayer 205 treatment only, as initial treatment.
(See pages 162 and 164)

Age group			0-5 years		6-15 years		Over 15 years		Total	
			Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Class 1	0	1	0	1	0	0	0	2
" 2	0	0	1	2	0	1	1	3
" 2a	1	0	1	2	0	0	2	2
" 3	0	0	0	0	2	2	2	2
" 3a	0	0	0	3	2	0	2	3
" 4	0	0	9	0	73	15	82	15
" 4a	0	0	0	0	4	4	4	4
" 5	0	0	0	0	3	0	3	0
Total	1	1	11	8	84	22	96	31

EXPLANATORY NOTES.

Class 1 = Cases receiving 1 gramme Bayer 205 in one injection.

" 2 = " " 2 " " " " 3-8 days.

" 2a = " " 2 " " " " " in a period of more than 8 days.

" 3 = " " 3 " " " " " 6-16 days.

" 3a = " " 3 " " " " " in a period of more than 16 days.

" 4 = " " 4 " " " " " 21-24 days.

" 4a = " " 4 " " " " " a period of more than 24 days.

" 5 = " " 5 " " " " " 24-30 days.

Relapses who are well after subsequent treatment are not included.

The deaths include cases who received treatment after relapse, with various drugs.

TABLE IV

Showing cases belonging to Category II treated with Bayer 205.
(See pages 162 and 164).

Age group			0-5 years		6-15 years		Over 15 years		Total	
			Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Class 3	0	0	0	0	1	7	1	7
" 3a	0	0	0	2	1	19	1	21
" 4	0	0	0	1	8	21	8	22
" 4a	0	0	0	2	3	7	3	9
" 5	0	0	0	1	1	0	1	1
Total	0	0	0	6	14	54	14	60

EXPLANATORY NOTES.

Classes as in Table III.

Relapses who are well after subsequent treatment are not included.

Deaths include cases who received treatment, after relapse, with various drugs.

TABLE V

Showing cases belonging to Category III treated with Bayer 205.

(See page 163).

Age group			0-5 years		6-15 years		Over 15 years		Total	
			Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Class 3	0	0	4	3	0	0	4	3
„ 3 ^a	0	0	0	2	1	3	1	5
Total	0	0	4	5	1	3	5	8

EXPLANATORY NOTES. As in Table III.

TABLE VI

Treatment with Tryparsamide alone.

A total dosage of at least 30 grammes was given in 2-4 gm. doses.

(See page 163).

			Category I	Categories II and III	Total
Well without further treatment	6	5	11
Relapsed re-treated—alive	8	6	14
dead	7	9	16
Total	21	20	41

EXPLANATORY NOTES.

Re-treatment *after relapse* included Bayer 205 as well as Tryparsamide.

TABLE VII

Showing cases belonging to Categories I, II and III, treated with Bayer 205 and Trypsamide.
(See pages 163, 164 and 165).

Age group	CATEGORY I				CATEGORY II				CATEGORY III				Total			
	0-5 years		6-15 years		0-5 years		6-15 years		0-5 years		6-15 years					
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead		
Class 6	2	1	1	2	2	11	0	1	0	7	12	1	1	1	15	30
"	0	0	4	1	16	5	0	0	1	8	11	0	1	3	33	19
"	0	0	2	2	4	3	0	0	0	3	11	0	1	0	9	18
"	0	0	2	2	11	0	0	0	1	8	15	0	0	1	22	19
"	0	0	0	0	16	11	0	0	1	11	13	0	0	2	29	30
"	0	0	1	0	30	7	0	0	1	24	23	0	0	2	56	32
Total	2	1	10	7	79	37	0	1	4	61	85	1	1	5	164	148

EXPLANATORY NOTES.

Class 6 = cases who received 1-2 grammes Bayer 205 and 2-11 grammes Tryparsamide as initial treatment.

[illegible]

Relapses, still alive, are not included.

Deaths include cases who were re-treated after relapse.

TABLE VIII

Showing cases treated with 4 grammes Bayer 205 followed by 31 or more grammes Tryparsamide.

(For particulars see text, pages 165 and 166).

Age group	Category I		Category II		Category III	
	Alive	Dead	Alive	Dead	Alive	Dead
6-15 years	0	0	1	0	0	0
Over 15 years	0	0	8	7	0	0

Among the dead are included three cases who relapsed and died after re-treatment.

TABLE IX

(See pages 165 and 166).

Showing cases treated with Bayer 205 and Tryparsamide in the following order :—

Bayer 205 1 injection

Tryparsamide 4 injections.

Bayer 4 „

Tryparsamide 8 „

(See text page 165 for details).

Category I		Category II	
Alive	Dead	Alive	Dead
12	1	13	7

All these were over 15 years of age.

Two cases not included in the table, belonging to Category II, relapsed and were re-treated.

TABLE X.

Showing the results of two different methods of treatment.

(See pages 165 and 173).

All these cases are over 15 years of age.

	Class	Well without further treatment	Relapsed re-treated alive	Relapsed re-treated died	Died without further treatment	Total
Category I	12	76	11	7	8	102
	13	15	3	2	2	22
Category II	12	9	5	6	15	35
	13	5	3	2	2	12
Category III	12	4	0	0	3	7
	13	0	0	0	1	1
Total	...	109	22	17	31	179

EXPLANATORY NOTES:—

Class 12 = Cases who received 4 grammes Bayer 205 over a period of 21-24 days, or 5 grammes over a period of 24-30 days.

Class 13 = Cases who received 4 grammes Bayer 205 and 22-30 grammes Tryparsamide over a period of 70-108 days, or 4 grammes Bayer 205 and 31-40 grammes Tryparsamide over a period of 84-128 days.

'Relapses' include possible re-infections.

Re-treatment in nearly all cases included both Bayer 205 and Tryparsamide.

TABLE XI

Showing action of Tryparsamide on cases which Bayer 205 failed to sterilise.
(See page 165)

Category	Case number	Amount of B. given	Period during which B. was given	C.S.F. at beginning of treatment	Interval between B. & T.	Condition during interval			Amount of T. given	Period during which T. was given	Observation period	Results				
			Days	Cells per c.mm.	Tryps.	Blood	C.S.F.		Days	Cells per c.mm.		Protein	Tryps.	General condition		
							Cells per c.mm.	Tryps.								
I	704	4 grammes	23	94	+	5	—	30	+	33 gms.	71	2½ years	1	Trace	N	Good
I	572	4 "	22	120	+	14	N	243	+	27 "	69	2½ "	1	—	N	"
II	756	4 "	22	249	+	121	+	—	—	*18 "	43	2 "	8	0.25%	N	"
II	578	4 "	22	90	+	13	+	113	N	27 "	41	2½ "	3	0.35%	N	"
II	Kalekwa	4 "	22	30	+	14	N	137	+	48 "	139	2½ "	1	0.16%	N	"

NOTES.

B = Bayer 205.

T = Tryparsamide.

+ = Positive.

N = Negative.

- = No observation.

C.S.F. = Cerebro-spinal fluid.

* = 1 gramme Bayer 205 given before Tryparsamide.

Interval between B. and T. refers to the interval between the last injection of Bayer 205 and the first injection of Tryparsamide.

TABLE XII

Showing the relationship between the duration of illness before treatment, the condition of the cerebro-spinal fluid, and oedema. (The oedema is most commonly found in the lumbar region, legs and feet).

All cases (except children who received proportionately smaller doses) received at least 3 grammes Bayer 205 and the majority at least 4 grammes. A few received Tryparsamide as well.

Duration of illness before treatment	0-7 days				8-14 days				15 days to 1 month			
	Negative		Positive		Negative		Positive		Negative		Positive	
	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema
Well, without further treatment	0	N	7	T	0	N	16	N	0	N	3	N
	0	N	30	+	3	N	23	N	0	T	3	N
	0	N	55	+	6	N	280	N	1	T	43	T
	0	N	80	-	13	N	340	+	2	N
	0	N	13	N	3	N
	1	N	13	N	3	T
	3	N	13	+	8	N
	3	N	20	N	9	N
	3	N	20	N	10	T
	3	N	30	N	16	+
	3	-	30	T	17	-
	3	T	33	N	23	-
	(a) 7	+	33	N	23	N
	10	N	33	N	25	N
	17	N	57	N	30	-
	20	N	60	N	30	N
	23	N	60	+	30	N
	23	N	63	N	30	N
	83	-	83	+	33	N
	240	N	37	N
	40	+
	50	N
	57	T
	67	+
Relapsed, still alive

Dead	(b) 0	+	(c) 3	N	20	N	3	N	73	N
	(c) 3	N	70	T	(f) 10	N	147	T
	(d) 10	N	15	N	447	N
	27	N
	110	T

SUMMARY.

Showing the relationship between the results of treatment and

(a) Cell content of the cerebro-spinal fluid ;

(b) Presence or absence of parasites in 2 drops of cerebro-spinal fluid in which the cell content was over 5 c.mm.

	Cells per c.mm.		Parasites in fluid	
	0-25	Over 25	Negative	Positive
Well without further treatment ...	52	34	48	16
Relapsed, still alive ...	1	8	4	5
Deaths ...	13	24	14	18
Percentage of recoveries excluding relapses who are still alive ...	80%	58.6%	77.4%	47%

TABLE XII—continued

Generally a fluid was labelled 'negative' when no parasites were found in two drops in a haemocytometer chamber.

In the table each reading for cells and oedema represents the condition of an individual case at the commencement of treatment.

(See pages 166 and 167).

Duration of illness before treatment	Over 1 month to 2 months				Over 2 months to 3 months				Over 3 months			
Tryps. in C.S.F.	Negative		Positive		Negative		Positive		Negative		Positive	
	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema	Cells per c.mm.	Oedema
Well, without further treatment	17	N	13	+	15	N	70	+	10	N	20	+
	23	N	30	+	40	N	507	N

	30	T
	50	N

Relapsed, still alive	70	+	20	N	67	+
	293	T	70	+	137	+
	90	+
	96	N
	120	N
Dead ...	3	+	13	T	60	N	50	N	30	+	13	++
	(g) 6	+	26	+	90	N	250	T	35	+
	20	N	30	+	50	+++
	(b) 40	N	87	N	90	+
	70	+	90	N	203	+
	93	+	120	+
	397	N

NOTES.

(a) = Old leper.

(b) = Oedema did not disappear with Trypanocidal treatment.

(c) = Cause of death unknown.

(d) = " " " "

(e) = Reported to have died of Dysentery.

(f) = Cause of death unknown.

(g) = " " " "

(h) = Albuminuria after Bayer 205, also Ankylostomiasis.

N. = Negative.

T. = Trace.

+

= Present.

- = No observation.

TABLE XIII
Showing the influence of intravenous injections of Bayer 205 on infected cerebro-spinal fluid.
(See page 168)

Condition of the cerebro-spinal fluid.

Case	Treatment	After 1st injection		After 2nd injection		After 4th injection		2-6 months after last injection		6-12 months after last injection		12-24 months after last injection		Over 24 months after last injection		Result.
		Cells per c.mm.	Tryps.	Cells per c.mm.	Tryps.	Cells per c.mm.	Tryps.	Cells per c.mm.	Tryps.	Cells per c.mm.	Tryps.	Cells per c.mm.	Tryps.	Cells per c.mm.	Tryps.	
734	4 B in 22 days ...	3	+	13	N	13	N	-	-	-	-	0	N	7	N	Well.
622	4 B " " ...	3	+	17	N	15	N	7	N	3	N	-	-	4	N	Well.
516	4 B " " ...	7	+	-	-	3	N	0	N	0	-	-	-	6	N	Well.
Wamala	4 B in 29 days ...	16	+	-	-	-	-	-	-	-	-	-	-	7	N	Well.
Mabula	4 B in 22 days ...	23	+	-	-	16	N	-	-	-	-	-	-	-	-	Well.
Ngelu	4 B " " ...	26	+	237	+	140	N	-	-	-	-	-	-	-	-	Died in a few months.
106	4 B " " ...	30	+	-	-	-	N	-	-	-	-	-	-	3	N	Well.
518	4 B " " ...	30	+	-	-	13	N	0	N	-	-	-	-	0	N	Well.
546	4 B " " ...	43	+	20	N	6	N	3	N	-	-	-	-	5	N	Well.
510	4 B " " ...	50	+	-	-	0	N	-	-	-	-	-	-	2	N	Well.
Lemi	4 B " " ...	50	+	-	-	0	N	-	-	-	-	-	-	4	N	Well.
Nangisa	4 B " " ...	53	+	-	-	56	N	-	-	-	-	-	-	-	-	Died shortly after treatment.
821	4 B " " ...	55	+	-	-	17	N	6	N	-	-	-	-	1	N	Well.
865	4 B " " ...	70	+	-	-	43	N	-	-	-	-	-	-	-	-	Died a year after.
567	4 B " " ...	80	+	30	N	10	N	10	N	-	-	-	-	3	N	Well.
660	4 B " " ...	90	+	247	N	53	N	-	-	-	-	-	-	-	-	Died a year after.
Mawe	4 B " " ...	143	+	-	-	-	N	-	-	-	-	-	-	-	-	Died a year after.
Kamila	4 B " " ...	203	+	60	N	-	N	63	N	-	-	-	-	-	-	Re-treated and died.
569	4 B " " ...	340	+	90	N	23	N	-	-	-	-	-	-	-	-	Died in a few weeks.
Munda	4 B " " ...	447	+	335	N	420	N	10	N	-	-	-	-	3	N	Well.
Katanga	4 B " " ...	120	+	20	N	-	N	136	N	-	-	-	-	-	-	Relapsed, re-treated, died.

NOTES.

N = Negative.
+ = Positive.
- = No observation made.

Observation period in cases who recovered was 29 months.
The injections were given weekly, except to Wamala, who missed one week.

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THE POLYMORPHIC TRYPANOSOMES OF DAMBA ISLAND, VICTORIA NYANZA

I.—THEIR ABILITY TO INFECT MAN

BV

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I. INTRODUCTION

The island of Damba lies between the Sesse and Buvuma groups in the northern waters of Victoria Nyanza. In former days, before the great epidemic of sleeping sickness swept through Busoga and Buganda in the early years of the present century, the island was thickly populated. Damba is covered from end to end with forest, and the shore, save where papyrus fills the bays or fringes the coastline, is very heavily infested with *G. palpalis*. At frequent intervals along the wooded coastline are the sites of the old villages, nestling in the dense forest, and others again are scattered through the forest but never very far from the water's edge. Conditions were indeed ideal for the rapid spread of human trypanosomiasis, and the people suffered very heavily before the survivors were removed to the mainland towards the end of 1909. While the island was still inhabited, the situtunga antelope, which occurred throughout the Sesse and Buvuma groups and also along the mainland coast, were strictly confined to the papyrus and grass swamps in which they

ordinarily find their home. Native evidence from pre-epidemic days is firm on this point.

In these swamps and in the narrow strip of short grass which often separates them from the edge of the forest behind, tsetse are seldom if ever to be found, and it may safely be assumed that native accounts are correct in stating that the antelope, which in the old days never left the swamps during daylight, seldom came into contact with *G. palpalis*. The flies of those days doubtless fed very eagerly on man, the numerous reptiles and hippo completing an ample dietary.

As the disease established itself on Damba and the population diminished, man gradually lost his ascendancy over his environment. Villages were abandoned, activity ceased and infected natives in all stages of the disease were constantly exposed to numerous tsetse, both in the villages and in the canoes along the water's edge. The fly on Damba at the time of the exodus in 1909 must have been infected to its utmost capacity with trypanosomes taken from man's blood.

After the evacuation of the island, the situtunga, freed from their principal enemy, began to multiply rapidly. They frequented the forest more and more as time went on, and their numbers increased to a point where their intensive grazing changed the very appearance of the coastline, the branches being nibbled off all along the forest edge to a height of four or five feet. As the antelope multiplied they came more and more into contact with tsetse, the fly following them into the forest and penetrating the island from end to end. At the same time, hippo and crocodiles also increased in numbers, but the enormous numbers of situtunga, grazing in the open for some hours after sunrise and emerging again several hours before sunset, were undoubtedly an attractive and easily accessible food supply for the fly. For some time before the removal of the islanders it is quite certain that ample opportunity occurred for the transference by the fly of trypanosomes from man to the antelope.

Experiments carried out at Mpumu in 1910 and 1911 showed that *T. gambiense* can survive in antelope for at least twenty-two months in a form transmissible by tsetse, so it is justifiable to conclude that, once introduced into the situtunga, this trypanosome would have an excellent chance of survival.

On the other hand, it is possible that either the cattle originally present on Damba, or the situtunga in their old swamp habitat, or both these species, may have harboured trypanosomes of the *T. brucei* group, non-pathogenic to man. We know now that "wild" strains of *T. brucei* are often comparatively harmless to cattle, and the fact that cattle flourished exceedingly on the Sesse Islands before and during the epidemic does not by any means exclude the presence of *T. brucei*. *T. gambiense* is also harmless to cattle and indeed to domestic animals generally.

There is then nothing in the available records of those early days to show whether or not *T. brucei* occurred on the northern islands of Lake Victoria. If it was present, the cattle must have been its principal, if not sole, host, for the antelope, as already stated, were protected from the fly by their habits and habitat. The cattle were, of course, all removed at the same time as their native owners.

The bearing of this retrospect becomes apparent when we consider the investigations of the tsetse and antelope on Damba Island carried out since the removal of the inhabitants. From January to June, 1910, i.e., a few months after the islanders had departed, the Royal Society's Commission at Mpumu fed 6,456 wild *G. palpalis*, caught on Damba Island, on seven monkeys. Two of these monkeys became infected with a polymorphic trypanosome, which the Commission considered to be *T. gambiense*. In May, 1911, Carpenter again recovered this trypanosome from 885 wild Damba tsetse. Later, in 1911, I isolated two strains of a polymorphic trypanosome from four situtunga shot on the island (referred to later on as the 1912 strain). These strains were carefully examined at the Mpumu laboratory and found to resemble *T. gambiense* rather than *T. brucei*. At the time, the importance of an accurate differential diagnosis was fully realised, and the animal reactions, external morphology and behaviour with human serum were the points studied. The view was put forward that the parasite was *T. gambiense* modified somewhat by two years' sojourn in antelope, out of all touch with man. (Duke, 1912.)

From 1911, similar trypanosomes have been recovered at intervals from either the *G. palpalis* or the situtunga on Damba. In 1920, twice in 1924, and again in 1926, visits to the island were successful. Then, after a series of failures, the reasons for which have been

discussed elsewhere (Duke, 1926), in February of the present year the three strains that form the subject of this paper were recovered from the wild fly on the island.

According to the view I was led to put forward in 1912, these three latest strains are the descendants of the human trypanosome, *T. gambiense*, that decimated the population of Damba twenty-five or so years ago. It was to be expected that the sojourn in antelope would sooner or later exert an effect on the attributes of the trypanosome, and signs of such modification were already discernible to the eye of faith in the 1920 strain when compared with that isolated in 1911. The characters of the three 1932 strains are now being carefully examined, and it is already evident that in one important respect at least they differ from the earlier strains, the change being still in the direction of a closer resemblance to *T. brucei*.

The possibility of the introduction of polymorphic trypanosomes from outside sources into Damba may be at once dismissed, and it is entirely reasonable to suppose that the strains of to-day are of the same stock as those recovered in former years.

The ability of these three recently isolated strains to infect man is therefore a point of considerable interest. First of all, the knowledge that the fly of the islands and the mainland coastline has carried, ever since the epidemic, a polymorphic trypanosome which bears a certain resemblance to *T. gambiense*, has always been an obstacle to the economic reclamation of this valuable territory. And again, from the biological standpoint, the experiments now to be described are complementary to the study of the morphology and reactions of these strains at present in progress at Entebbe. Whether the results described below apply to all strains of apparently identical trypanosomes in the lakeshore fly and antelope, and how far the volunteers employed are representative of mankind in general, are not points of great practical importance.

The present investigation supplies a finishing touch to the huge 'Fishermen Experiment' commenced in 1922 at the instigation of Mr. W. F. Fiske, while Reclamation Officer to the Uganda Government.

In a future publication, these 1932 strains will be fully described and contrasted with those obtained in former years.

II. THE VOLUNTEERS

Research into human trypanosomiasis has now reached a stage where further progress in certain important directions necessitates the employment of man as an experimental animal. Under the aegis of the British Government, the employment, in any circumstances whatever, of criminals, condemned or otherwise, for purposes of this kind is out of the question. The use of European volunteers is a matter for mutual arrangement. A good cause frankly explained will always find the support it deserves. The enrolment of native volunteers requires, however, some explanation.

As a tribe, the Baganda of to-day are very much more sophisticated than most of their neighbours. They do not indulge in any form of voluntary mutilation of their persons, as do many of the east and central African tribes, and they are extremely averse from tampering in any way with their health. Moreover, the native staff of the laboratory have long since acquired a wholesome respect for tsetse, whether wild or in the experimental boxes which they handle in the course of their daily duties.

I had, therefore, but slender hope of securing any native volunteers for the present series of experiments, the more so as one of the 'fly boys' within recent years had accidentally become infected with *T. gambiense*, through careless handling of a box of tsetse at the laboratory, and though quickly cured underwent a certain amount of inconvenience before treatment was commenced.

To be brief, I explained fully in the vernacular to the assembled staff the object as well as the risks of the experiment, offering a reward to anyone who would come forward. That one and all understood the drift of the address was shown by the spokesman's summary of my appeal: 'You wish to know whether our blood will destroy the little insects that have killed those monkeys we brought back from Damba.' No form of compulsion was applied, direct or indirect, and they were given twenty-four hours to deliberate. The next day three of them volunteered; one has been with me since the Mpumu days in 1910, and the other two are also employees of long standing. Later on, others came forward and were inscribed on the roll of service.

The intended course of events was disturbed by a number of minor

incidents. First, one of the volunteers, by a foolish indiscretion, incurred a sentence of nine months' imprisonment, and disappeared from the scene before the experiment started. His place was eagerly filled by another candidate. Then, after having been fed upon by four boxes of tsetse, another of the original volunteers developed a mild attack of chicken-pox and was taken off to the isolation hospital, where he duly fulfilled his share of the experiment. And finally, the original leader, in a moment of depression on the eighth day of his service, sought solace and oblivion in native beer, and arrived at the laboratory next day in a parlous condition. These 'debilitating influences,' as will be seen, exercised no apparent effect on the course of the experiment. Of minor interest is the observation that blood of the chicken-pox subject (N.P., of a list given below), at the height of the attack, had no effect on the flagellates in the infected flies that took up his blood.

In the Tables, the European volunteer is denoted by E, the three natives are shown as N.M., N.P. and N.E., respectively.

In these experiments it was not possible to say beforehand whether any given box of flies was infective. To find this out would have entailed a serious risk of the loss by death of infective flies before they had bitten the volunteers. In consequence, a certain number of negative boxes were used in addition to those described in Table I. The total number of flies biting each volunteer were as follows :—

N.M., 329. N.P., 373. N.E., 421. E., 157.

A word may be said about the effect of the flies' bites on the human skin. In the case of the European this was easily observed. All the three boxes that fell to his share were fed upon the same area of skin on the forearm. On removal of the box after the first feed, a number of drops of the clear solution exuded by a full-fed fly after its meal were seen on the skin. Some of these droplets were bloodstained by petechial haemorrhages through the subjacent bite. The next day the skin showed a number of small areas of discolouration exactly like bruises. All over the area were mildly irritating papules, which soon disappeared with iodine and hot water. The next box, put on the same area a few days later when the bruising had almost disappeared, produced no more bruising and much less irritation. The irritation after the third box was also slight. A control

'clean' box, fed on the same part of the other forearm, produced another crop of bruises and papules. There is here a suggestion of acquired skin immunity, which it may be convenient to investigate further at some future date.

III. ORIGIN OF THE 1932 STRAINS

The three strains that form the subject of the paper were recovered by feeding on clean monkeys wild *G. palpalis* caught on the shore of Damba Island. The site chosen for the camp on the island was a new one, and the flies were caught along a stretch of shore not examined during the last eight years. All the three monkeys were infected by the first batches of flies fed upon them, i.e., some 300 flies in each case. The incubation period in each case was six to seven days and during this period each monkey was bitten by some 2,000 flies in all. No fly fed on more than one monkey. Each animal thus represents at least one and possibly more wild strains. A fourth monkey was inoculated subcutaneously with defibrinated blood from six situtunga without becoming infected.

In what follows, the three strains are distinguished by the experimental number of the monkey in which each reached the laboratory, i.e., 910, 911, 912, respectively. Of the three strains, 912 proved the most readily transmissible by *G. palpalis*.

IV. TECHNIQUE

Laboratory bred *G. palpalis* were fed on the infected monkeys and then nourished by feeds on alternate days on a clean monkey or a fowl. After twenty-five days or so the flies were fed upon the clean animals which it was desired to infect. As will be seen in Table III, the infectivity of the flies was tested before and after their meal on man. A day of starvation, or in some cases two days, alternated with each feed. In the great majority of cases every fly in an experimental box fed readily, but in the case of the all-important feeds on the volunteers each box was kept on until no more flies bit, and replaced, if necessary, later on should any fly appear not to have fed. Lastly, in the case of one or two individual flies which obstinately refused to take up blood from the volunteer, the unfed insect was removed from the box and dissected at once. It can

therefore be stated beyond all shadow of doubt that all the flies dissected at the end of each experiment had in truth fed upon the volunteer concerned.

At the end of each experiment the surviving flies were killed with chloroform vapour and dissected. The degree of infection with flagellates is roughly indicated by the ' + ' symbol; ' + + + gut ' meaning a fully infected gut with glands uninfected; ' + + + gut and gld.', a fly with a fully developed gland infection. The symbol (+) after an animal's designation shows that the animal developed trypanosomes as the result of its treatment. *Box* = a collection of about 50 laboratory bred *G. palpalis*, which constitutes a single experiment; *M* = monkey; *S* = sheep; *std.* means that the box was starved.

V. THE TRANSMISSION EXPERIMENTS

The actual transmission experiments will now be presented. From the twenty-fifth day of an experiment onwards, a fly, if it is ever going to become infective, will have trypanosomes in its salivary glands. Before that date the boxes were fed on monkeys or fowls, which, as will be seen in Table I, remained negative.

In these investigations, the number of animals available for demonstrating the infectivity of the flies was not adequate to test each individual box both before and after the feed on man. The Tables show, however, that each volunteer was indeed bitten by flies that had undergone the full double test. It must be remembered, too, that an infective fly need not take up an obvious amount of blood in order to produce infection in the animal it bites. It was shown years ago at Mpumu that the mere act of inserting the proboscis is sufficient to infect, provided the glands are well infected with trypanosomes.

VI. DISCUSSION

The realisation that the Damba trypanosome, and, in all probability, the similar strains carried by *G. palpalis* along the whole northern littoral of Lake Victoria, are innocuous to normal man simplifies considerably the task of reclaiming this fertile territory.

Attention can now be concentrated wholly on keeping out the human reservoir of the disease in the shape of infected natives

from neighbouring foci of human trypanosomiasis. There are, or were up till quite recently, such foci to the east of Uganda. That they are a genuine source of danger is shown by the crop of cases that developed a few years ago at Port Bell, near Kampala. Here the outbreak was easily traced to an infected native from the north-east corner of the Lake.

From the biological standpoint, these experiments do not help in the elucidation of the origin of the wild fly and antelope trypanosomes of Damba and the adjoining littoral. The results obtained are equally consistent with a belief in an unmodified *T. brucei* or a modified *T. gambiense*.

In a recent paper on the action *in vitro* of normal human serum on trypanosomes, Yorke, Adams and Murgatroyd refer to my views on these lake-shore trypanosomes, remarking as follows:— 'It is true that Duke produces some evidence that the 1920 antelope strain was more virulent for monkeys than was the 1912 antelope strain; but we cannot but be impressed with the fact that the differences in pathogenicity between the 1912 and 1920 antelope strains appear, according to Duke's own data, to be distinctly less than those between the 1912 antelope strain and the strain of *T. gambiense* derived from man.' (Yorke, 1930.) This quotation calls for brief comment.

First of all, the *T. gambiense* strain to which it refers was isolated about 1920 from a native from the Mpologoma area of the Eastern Province of Uganda. In this area human trypanosomiasis has persisted in man since the early days of the epidemic. The disease is of a chronic type. It was in this eastern infected area that Van Hoof in 1926 found evidence of a case of auto-recovery; and the strains in this region in 1920 were very different from those occurring along the Buganda lake-shore at the height of the epidemic, some of which were remarkably virulent in man (cf. Duke, 1921).

There is, moreover, another consideration which seems to me to affect the criticism. The theory under discussion is that the *T. gambiense* on Damba Island, on the disappearance of man, adopted the situtunga as its principal mammalian host, and has survived in the antelope, with certain modifications, until the present day. On this assumption, the greatest changes in the trypanosomes will surely be associated with the actual transition period, that is to

TABLE I. Showing the protocols of the
The different experiments were started on different days on the infecting monkeys, and a number of other boxes

Day of Experiment	531	534	537	538	
1	M 912	M 910	M 912	M 912	
2	std.	M 910	std.	std.	
3	M 912	std.	M 912	M 912	
4	std.	Fowl 9	std.	std.	
5-23	M 880 and Fowl 20		M 919 and Fowl 2	Fowl 2	
24					std.
25					M 930 (+)
26					std.
27	std.	♀ dies, gut + + +, gland + +	M 929 (+)	M 929 (+)	
28	std.	N.M.	std.	std.	
29	N. P.	std.	std.	std.	
30	std.	♂ 25, ♀ 19 surviving flies dissected. ♀ 1 + + + gut and glands. Gland inoculated into M 932 (+). NOTE.—This box infected M 930. It is impossible to say if the fly that died on 27th day bit M 930.	N. P.	S 46 (+)	
31	♂ 15, ♀ 13 surviving flies dissected. ♀ 1 + + + gut; glands infected in ducts only. NOTE.—This fly did not infect M 929; on the 26th day of the experiment, the glands were in all probability not infective. It may however have introduced trypanosomes into N.P. three days later.		♂ 22, ♀ 21 surviving flies dissected. ♀ 1 + + + gut and glands. Glands inoculated into M 933 (+). NOTE.—Boxes 537 and 538 both fed upon M 929 on same day.	std.	
32				S 46 (+)	
33				std.	
34				N. M.	
35				std.	
36				E	
37				std.	
38				N. E.	
39				♀ 18, ♀ 30 surviving flies dissected. ♀ + + + gut and glands. Glands inoculated into M 945.	
40					

positive boxes fed upon the volunteers.

were employed, but are not included here as they contained no flies with infected salivary glands.

543	544	545	553	558
M 912	M 912	M 910	M 910	M 911
std.	std.	std.	std.	std.
M 912	M 912	M 910	M 910	M 911
std.	std.	std.	std.	std.
M 919	Fowl 8	Fowl 15	Fowl 19	Fowl 23
std.	std.	std.		
M 929 (+)	M 929 (+)	M 930 (+)		
std.	std.	std.	std.	std.
S 44 (+)	S 44 (+)	N. M.	M 935 (+)	M 934
std.	std.	std.	std.	std.
N. P.	N. P.	♂ 30, ♀ 23 surviving flies dissected. ♀ 1 +++ gut and gland. Glands inoculated into M 932 (+).	N. E.	N. E.
std.	std.	NOTE.—Fed on M 930 four days later than Box 534. The monkey was then infected, but no trypanosomes were discernible in its peripheral blood.	std.	std.
♂ 15 surviving flies dissected. ♂ 2 and ♀ 2 +++ gut and glands. Glands inoculated into M 938 (+). NOTE.—Boxes 543 and 544 fed on M 929 the day following Boxes 537 and 538.	♂ 20, ♀ 16 surviving flies dissected. ♂ 3, ♀ 1 +++ gut and glands. Glands inoculated into M 939 (+).		N. P.	♂ 18, ♀ 13 surviving flies dissected. ♂ 1 +++ gut and glands. Glands inoculated into M 944 (+). NOTE.—M 934 never became infected. Presumably the infected fly did not bite the monkey.
			std.	
			E	
			std.	
			Fowl 19	
			std.	
			M 920 (+)	
			♂ 27, ♀ 20 dissected, ♂ 1 +++ gut and glands.	

TABLE II.

Showing the extent to which each volunteer was exposed to flies known to be infective with the *Damba* trypanosome.

Volunteer	Strain of trypanosome	Date when flies bit volunteer	Experimental box used	Number of flies with infected salivary glands biting volunteer	Proof of infectivity of flies before or after biting volunteer	Remarks
N.P.	912	27.3.32	531	1	None	Only the salivary ducts of this fly infected.
N.P.	912	30.3.32	537	1	Before and after	...
N.P.	912	1.4.32	543	4	} Before and after	...
N.P.	912	1.4.32	544	4		
N.P.	910	6.4.32	553	1	Before and after	...
N.M.	910	27.3.32	534	1	Before and after	...
N.M.	910	30.3.32	545	1	After	...
N.M.	912	3.4.32	538	1	Before and after	...
N.E.	910	4.4.32	553	1	Before and after	...
N.E.	911	5.4.32	558	1	After	...
N.E.	912	7.4.32	538	1	Before and after	...
E.	912	5.4.32	538	1	Before and after	...
E.	910	8.4.32	553	1	Before and after	...

TABLE III

Showing the manner of employment of the animals used as indicators of the infectivity of the flies before and after biting man.

Animal	Date		Incubation period in days	Strain of trypanosomes	Mode of infection	Proving infectivity of flies before or after biting volunteers	Remarks
	of infection	of first appearance of trypanosomes in blood					
M 930	24.3.32	2.4.32	9	910	Flies of box 534	Before	...
M 932	29.3.32	5.4.32	7	910	Inoculation of gland from box 534	After	...
M 929	27.3.32	5.4.32	9	912	Flies of box 537	Before	...
M 933	31.3.32	12.4.32	12	912	Inoculation of gland from box 537	After	...
S 46	30.3.32	8.4.32	9	912	Flies of box 538	Before	...
M 945	8.4.32	16.4.32	8	912	Inoculation of gland of box 538	After	...
S 44	30.4.32	7.4.32	8	912	Flies of box 543	Before	...
M 938	3.4.32	12.4.32	9	912	Inoculation of glands of box 543	After	...
M 939	3.4.32	21.4.32	18	912	Inoculation of glands of box 544	After	A delay in the inoculation of these glands led to attenuation of inoculum
M 935	2.4.32	12.4.32	10	910	Flies of box 553	Before	M 920 a feeble animal.
M 920	12.4.32	18.4.32	6	910	Flies of box 553	After	This fly had failed previously, presumably through not biting, to infect M 934
M 944	7.4.32	17.4.32	10	911	Inoculation of gland of box 558	After	

say, while the parasite was still engaged in adapting itself to its new host. By the end of 1911, already two years had elapsed since the departure of the islanders—two years during which *T. gambiense*, for the first time perhaps for centuries, found itself liberally provided with an easily accessible mammalian host that was supremely indifferent to its presence in the bloodstream. That the alleged changes were in the direction of the *T. brucei* type fits in with the hypothesis advanced in 1921 and expressed as follows:—‘ . . . the polymorphic trypanosomes of Africa all belong to a single species and not to a multitude of species . . . Included in this species are many different strains. . . . These strains are not immutable but variable and are determined by the environment in which the species lives; they are not constant under varying external conditions, but each different strain is dependent upon, and is produced as a response to, a particular environment. If we call this species *Trypanosoma brucei*, then “*T. gambiense*,” “*T. rhodesiense*,” “*T. nigeriense*” are to be regarded as particular strains of *T. brucei*, which have become, after sojourn in other hosts, more or less adapted to life in the blood of man.’ (Duke, 1921.)

There is as yet no unequivocal evidence that a trypanosome recovered from man's blood may lose its power of infecting man. Whether or not this happens in nature is the main problem of trypanosomiasis research to-day. For my part I do not believe that the *in vitro* technique devised by the Liverpool workers, in its present form at any rate, can be regarded as infallible in settling this point. In this connection Corson's recent work is of great interest, and I have to thank him for allowing me to see the manuscript of his paper now in the press at Liverpool. Corson infected himself by syringe inoculation with a strain of *T. rhodesiense* which had been maintained by direct passage in goats, sheep and rats for nineteen months and then passed once cyclically through tsetse. A month or so before the human experiment, this strain had been examined by Dr. Adams at Entebbe and found to be sensitive *in vitro* to undiluted normal human serum. Corson himself showed that it was also sensitive, *in vivo*, to his own and to other normal human sera. Again, Adams found that trypanosomes, unaffected *in vitro* by baboon's serum, when inoculated into the baboon produced no demonstrable infection.

And lastly, according to experience acquired by the study of *T. rhodesiense* when exposed to human serum *in vitro*, it would appear that this trypanosome loses its resistance very rapidly when once removed from man. Corson's experience, single experiment though it be, is at variance with this conclusion. Unless Corson's own serum is deficient in trypanolytic substance—and he has shown it to be efficacious *in vivo* against both *T. rhodesiense* and Hornby's strain of *T. brucei*—he has successfully demonstrated that *T. rhodesiense* can retain its power of utilising man for at least nineteen months. Unpublished work by Adams at Entebbe makes it exceedingly improbable that the single cyclical passage of Corson's strain through tsetse had any effect on its pathogenicity to man.

There seem, therefore, to be discrepancies between the results obtained *in vitro* and *in vivo*, which suggest that some other, as yet unknown, factor participates in the determination of the success of a trypanosome in nature.

But concerning the main problem before us, namely, whether in any circumstances obtaining in nature *T. rhodesiense* or *T. gambiense* do lose their infectivity for man, Corson's results, as he himself readily admits, do not help us. His *T. rhodesiense* strain killed goats regularly in about three weeks, and could therefore never hope to survive in nature in these animals. Moreover, in so sensitive a host, the trypanosome is not subjected to the restraining and adjusting influences which it will presumably meet in the blood of the far more resistant antelope.

To answer the question still outstanding, the trypanosome must be transferred from man through a series of antelope by cyclically infected tsetse—I do not think it matters what species of tsetse is used—the passages being effected preferably at long intervals so that the full effect of each animal's reacting power may be exerted on the trypanosome. And the last host in the experimental series will have to be the human volunteer.

VII. SUMMARY

Laboratory bred *G. palpalis* cyclically infected with three different strains of polymorphic trypanosomes recovered from the wild *G. palpalis* on Damba Island, Victoria Nyanza, have been fed upon

four normal human beings, three natives and one European. One native was in this way exposed to infection by all three strains, the other three volunteers to two strains each. None of the volunteers became infected.

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YELLOW FEVER TRANSMISSION EXPERIMENTS WITH SOUTH AMERICAN BATS*

BY

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R. M. Gordon (1922), in Manàos, Brazil, found that *Aedes aegypti* fed on bats with great readiness and voracity, usually piercing the wing membranes of the animals. He suggested that bats might serve as important food reservoirs for these mosquitoes in deserted houses or sparsely inhabited districts. When in January, 1930, the writer met Dr. Gordon at the Sir Alfred Jones Research Laboratory in Freetown, Sierra Leone, Dr. Gordon pointed out to him the importance of making yellow fever transmission experiments with bats. Because of the great numbers of these animals in Northern Brazil and elsewhere, and because of Dr. Gordon's observation that *Aedes aegypti* readily fed on them, it seemed of practical value to determine whether bats could serve as a reservoir of yellow fever virus.

Gordon (1922) found six species of bat common in and around houses in Manàos. Two of these species have also been found by us in Bahia, namely, *Molossus obscurus*, Geoffroy, and *Molossus rufus*, Geoffroy. With *Molossus obscurus* captured in that city, we were able to confirm Gordon's finding that *Aedes aegypti* fed readily on this bat. On several occasions large numbers of *Aedes aegypti* mosquitoes took blood from *Molossus obscurus* bats when the latter were placed in cages containing unfed mosquitoes. We had hoped to conduct yellow fever transmission experiments with *Molossus obscurus*, but we were not again able to obtain this species in large numbers.

Molossus rufus appeared to be more common in Bahia than *Molossus obscurus*; and as *Aedes aegypti* fed just as readily on the one as on the other, *Molossus rufus* was used in the experimental

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transmission work. On two occasions, more than fifty *Aedes aegypti* mosquitoes took blood from single specimens of *Molossus rufus* in about half-an-hour. Each bat was put in a cage with about 180 unfed *Aedes aegypti* mosquitoes, and the insects attacked the bat almost immediately. Transmission experiments were also made with *Desmodus rotundus*, Geoffroy, a common vampire bat of South America on which *Aedes aegypti* will feed, though not so readily as on *Molossus obscurus* or *Molossus rufus*. Specimens were brought to the yellow fever laboratory from the city of Bahia and also from Bananeiras, a small settlement in the interior of the state.

Great difficulty was experienced in inducing *Molossus obscurus* and *Molossus rufus* to feed while in captivity. At least six different fruits, numerous live insects, milk, bread and chopped meat were tried, but the bats showed no interest in any of them. Occasionally they would nibble at ripe bananas, but as a rule they all died of starvation within a week or so. The period between the time when the bats were first fed on by infected *Aedes aegypti*, and the time when they were either fed on by normal mosquitoes or bled for injection of their blood into normal monkeys, is here called the 'period of observation.' By using freshly caught bats and confining this 'period of observation' to not more than seven or eight days, it was possible to carry out the experiments with *Molossus rufus*.

Desmodus rotundus fed more readily in captivity, both on bananas and on live animals. Two adults of this species were kept alive for twelve days by allowing them to take blood every day or two from a live monkey or guinea pig. Presumably they could have been kept alive much longer, but the process was rather costly in guinea pigs, as a guinea pig usually died from exsanguination after being fed upon once by the vampire bats. Even *Macacus rhesus* monkeys lost a great deal of blood, and their temperature fell to subnormal after being bled by one or two of these bats.

Two types of experiments were tried: transmission of yellow fever virus through the bats by means of mosquitoes, and transmission by the bites of the vampire bats. The former would appear to be the more important possibility, epidemiologically. The procedure employed was first to allow *Aedes aegypti* mosquitoes known to be infected with yellow fever virus to feed on the bats, then to keep the bats over a 'period of observation' of four to

eight days, and finally to allow normal *Aedes aegypti* to feed on them to see if the mosquitoes would again pick up the virus. This was done in six experiments with *Molossus rufus* and three experiments with *Desmodus rotundus*. The experiments with these two species of bat are recorded in Table I.

The results appeared to be definitely negative in experiments 1, 4, 5 and 6 with *Molossus rufus*, and in all three experiments with *Desmodus rotundus*. Experiments 2 and 3 were inconclusive, because the monkeys finally fed on by the mosquitoes showed no reaction on retest inoculation. However, the source of virus for these two experiments was not properly controlled, and their significance is accordingly diminished. It would seem that bats would be of importance as reservoirs of yellow fever in nature only if they could be infected by *Aedes aegypti* and if normal *Aedes aegypti* could later obtain the virus from them again. This did not occur.

Several other species of bat are readily captured in Bahia, but the experiments were not pursued further because of the consistently negative results obtained with the species tried. *Molossus rufus* seemed to be the commonest species found in deserted houses in the city, although considerable numbers of vampire bats were captured in such houses on one or two occasions.

Transmission of yellow fever virus by the bite of the vampire bat was attempted in two ways. (1) Bats which had been fed on by infected lots of mosquitoes were used. If they had been susceptible to yellow fever on being bitten by mosquitoes, or had taken the virus into their general circulation, the possibility that they might excrete the virus in their saliva when biting a normal monkey was considered. Four *Desmodus rotundus* bats which had been fed on by infected lots of *Aedes aegypti* two days previously were allowed to bite normal *Macacus rhesus* No. 1. They fed readily for about an hour. Two days later three of the same bats again took blood from *Macacus rhesus* No. 1. The monkey lost so much blood at this second feeding by the vampire bats that on the following day its temperature fell to 97° F. But it later recovered and never showed any abnormal elevation of temperature. Retest inoculation of yellow fever virus of the Asibi strain produced a two-day fever, with eventual recovery. The virus used for the retest inoculation was unfortunately not very potent, as only one of the four presumably

TABLE I

Attempts to transmit Yellow Fever to Bats by infected *Aedes aegypti*.

Experiment number	Species of bat and number used	Strain of virus used	Mosquito source of virus definitely shown to be infected	'Observation period' in the bats (days)	RESULTS				
					Result of inoculation of blood drawn from the bats into a normal monkey	<i>Aedes aegypti</i> lot number fed	Incubation period in this lot (days)	Result of feeding this lot on a normal monkey	Result of injecting this lot into a normal monkey
1	<i>Molossus rufus</i> (1) ...	Asibi ...	Yes	4	Fever due to tuberculosis, but monkey died of yellow fever on retest inoculation.*	509	16	No fever, but monkey died of yellow fever on retest inoculation	No fever, but monkey died of yellow fever on retest inoculation
2	<i>Molossus rufus</i> (1) ...	Asibi ...	Yes ? (No control test)	4	No fever, but monkey died of yellow fever on retest inoculation.†	510	16	No fever and no reaction to retest inoculation	
3	<i>Molossus rufus</i> (1)	Asibi ...	Yes ? (No control test)	4	Fever due to tuberculosis, but monkey died of yellow fever on retest inoculation.*	511 ‡	16	No fever and no reaction to retest inoculation	
4	<i>Molossus rufus</i> (1) ...	Asibi ...	Yes	4	No fever, but monkey died of yellow fever on retest inoculation.†	512 ‡	16	No fever and no reaction to retest inoculation	No fever, but monkey died of yellow fever on retest inoculation
5	<i>Molossus rufus</i> (4) ...	A.S.C.	Yes	7		545	14	No fever, but monkey had fever with eventual recovery on retest inoculation	
6	<i>Molossus rufus</i> (4) ...	A.S.C.	Yes	8		546	14	No fever, but monkey died of yellow fever on retest inoculation	
7	<i>Desmodus rotundus</i> (1)...	Asibi ...	Yes	6		552	15	No fever, but monkey died of yellow fever on retest inoculation	
8	<i>Desmodus rotundus</i> (1)...	Asibi ...	Yes	6		552	15	No fever, but monkey died of yellow fever on retest inoculation	
9	<i>Desmodus rotundus</i> (3)...	Asibi ...	Yes	6		553	15	No fever, but monkey died of yellow fever on retest inoculation	

* The blood drawn from bats 1 and 3 was inoculated into the same monkey.

† The blood drawn from bats 2 and 4 was inoculated into the same monkey.

‡ Mosquito batches 511 and 512 were fed on the same monkey.

|| The same four bats were used in experiments 5 and 6.

susceptible monkeys inoculated at the same time as *Macacus rhesus* No. 1 died of yellow fever. There seemed to be no indication, however, that any virus was secreted in the saliva of the vampire bats. (2) Direct mechanical transmission was tried in three experiments, using two different strains of virus, the A.S.C. and the A.W.B. strains. The A.S.C. is probably modified Asibi, or West African virus, while the A.W.B. is probably a South American virus. Fatal yellow fever was produced in both cases upon immediate transfer, as is shown in Table II.

TABLE II
Mechanical transmission experiments with *Desmodus rotundus*

Experiment Number	Strain of virus used	Temperature of source monkey and day of fever	Number of vampire bats used	Time between feeding on the source monkey and on the test animal	Result in test monkey
10	A.S.C.	104° F. 2nd day of fever	2	About 1 minute	Fatal yellow fever
11	A.W.B.	105° F. 1st day of fever	3	About 1 minute	Fatal yellow fever
12	A.S.C.	104° F. 2nd day of fever	2	About 20 minutes	No fever, but on re-test inoculation monkey had fever, with recovery

The following procedure was used. The bats were kept in individual cages and allowed to feed one after another. First, the source-of-virus animal was put into each cage. The bats were pretty well starved and very soon started to feed. They were allowed to take blood for about five minutes, and then the source-of-virus monkey was removed and was replaced by a normal animal. The bat completed its blood meal on the normal animal. In the two experiments, in which not more than a minute or two elapsed between the feeding of the bats on the infected monkeys and on the normal animals, fatal yellow fever was produced in the latter. In the third experiment, No. 12, the test animal showed no reaction, but it did not die on retest.

It is interesting to note that mechanical transmission of yellow fever virus can be obtained by the bite of South American vampire

bats, but it is probably of little or no epidemiological importance. Such a mode of transfer might occur if the virus were circulating in susceptible wild South American monkeys, but it seems unlikely in human cases. As to other diseases possibly transmitted by bats, Hurst and Pawan (1931), in describing an outbreak of rabies in Trinidad without history of bites, suggested that the virus was perhaps transmitted by vampire bats, because they had noted that, when cattle were moved from pastures to barns in which lanterns were hung to scare away the bats, the spread of the disease among the animals was arrested. They quoted Haupt and Rehaag (1921), who had reported a severe epidemic of rabies in cattle and horses in Brazil, which they said was due to the bite of the vampire bat *Phyllostoma superciliatum*, Burmeister.

Since vampire bats can transmit yellow fever mechanically, the question of mechanical transmission by insects again arises. Up to the present time, mechanical transmission of yellow fever virus by insects has not been obtained, although certain other diseases, presumably also caused by filtrable viruses, have been transferred in this manner. Philip (1930), in West Africa, made mechanical transmission experiments with yellow fever virus, using the insects *Aedes aegypti* and *Cimex lectularius*. His results were negative. However, Simmons (1931) reported, on the basis of experiments made in the Philippine Islands, that the virus of dengue had been transmitted mechanically from man to man by *Aedes aegypti* and also by *Culex quinquefasciatus*. Kligler, Muckenfuss and Rivers (1929) transmitted the virus of fowl-pox mechanically by *Culex pipiens*, using periods as short as fifteen minutes between feedings, and it is well known that certain diseases of animals may be transmitted mechanically by species of *Glossina* and *Stomoxys*, and by some of the *Tabanidae*.

In one experiment we repeated the work of Philip in West Africa, and also obtained negative results. Seventy-five *Aedes aegypti* were used in individual tubes. Each mosquito was separately observed to engorge partially on an infected monkey. Its feeding was then interrupted, and it was immediately transferred to a normal monkey and allowed to complete its blood meal. The second monkey showed no fever. After a period of twenty-one days, these same mosquitoes were again allowed to feed on the same normal monkey. As a result of the second feeding, the animal

showed fever for three days but eventually recovered. The experiment confirmed the observations of Philip, that immediate mechanical transmission of yellow fever by *Aedes aegypti* does not occur, but that the same batch of mosquitoes, after a suitable incubation period, can convey the disease.

SUMMARY

1. *Aedes aegypti* mosquitoes were observed to feed readily on both *Molossus obscurus* and *Molossus rufus*.

2. Attempts to transmit yellow fever virus to *Molossus rufus* and *Desmodus rotundus* by infected *Aedes aegypti* mosquitoes, and later to reobtain the virus from the bats by means of normal *Aedes aegypti* or by blood subinoculation, were negative in result.

3. Immediate mechanical transmission of yellow fever was obtained in two experiments by the bite of the common South American vampire bat *Desmodus rotundus*.

4. Mechanical transmission of yellow fever by the vampire bat is probably of little importance in the epidemiology of the disease in human beings. But mechanical transmission of certain diseases of animals by the bite of *Desmodus rotundus* or other vampire bats may be of considerable economic importance.

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STUDIES IN CHEMOTHERAPY*

VII. IS THE RESISTANCE OF A DRUG-FAST TRYPANOSOME MODIFIED BY TRANSFERENCE TO A DIFFERENT SPECIES OF VERTEBRATE HOST ?

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In almost all the recent reviews and essays on the subject of chemotherapy, particular attention is drawn to observations made many years ago by Mesnil and Brimont (1908), by Moore, Nierenstein and Todd (1908), and by Breinl and Nierenstein (1908). These observations were broadly to the effect that a strain of trypanosomes made resistant to atoxyl in one species of animal loses that resistance if transferred to another host. It is obvious that these observations must of necessity exert a profound influence on the development of any hypothesis to explain chemotherapeutic processes ; and it is consequently not surprising that all those, who have of late years troubled themselves with speculations regarding the mechanism of chemotherapeutic action, should have attached great weight to them, and that their lines of thought should have been in no small measure governed by them. That this, in fact, has been the case is shown by the following quotations :—

Dale (1923) writes :—

‘ A still greater complication is introduced by the behaviour of a resistant strain when transferred to a host of another species. Mesnil and Brimont produced a strain resistant to atoxyl in the mouse, and found that, when it was transferred to the rat, the sensitiveness re-appeared and the strain remained normally sensitive during forty passages through this host-species, to regain its resistance immediately when re-transferred, without further treatment, to the mouse. In the dog, on the other hand, the resistance acquired in the mouse was retained. There could be no clearer evidence of the co-operation of the host's tissues in the curative action of atoxyl ; on the other hand, it becomes very difficult even to speculate on the mode of their intervention.’

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Voegtlin, Dyer and Miller (1924) write :—

‘These and some other observations sufficed to demonstrate that Ehrlich’s original conception was far too simple to explain the intricate mechanism of chemotherapeutic action and the cause of drug-resistance. Ehrlich failed to give sufficient consideration to the *fate of drugs in the body* of the host. Nothing could demonstrate this function of the host more clearly than the following observations of Mesnil and Brimont (1908). They produced a trypanosome strain resistant to atoxyl in mice. This same strain when tested as to its arsenic resistance in rats showed a very low resistance only slightly above that of the original “normal” strain. The resistant strain after having passed through forty-three rats (one hundred and fifty-seven days) was then inoculated into mice and its arsenic resistance was found to be the same as before its transfer to rats.’

Wenyon in his manual on Protozoology (1926) writes :—

‘Mesnil and Brimont (1908) have shown, however, that a race which had become resistant to atoxyl, and had maintained this resistance when passed through mice, lost it when transferred to the rat, only to regain it when again passed into the mouse. It is evident that the tissues of the host play a part in the therapeutic process.’

In Findlay’s “Recent Advances in Chemotherapy” (1930) the following passage occurs :—

‘This lack of specificity is somewhat difficult to reconcile with the theory of specific chemoreceptors, as is the fact, first noted by Mesnil and Brimont (1908), that the arsenic resistance of a particular strain of trypanosome passaged in the mouse is lost if the strain is passaged in the rabbit or dog, but is regained when returned to the mouse. This fact suggests that the host’s tissues play some part in the development of the resistance.’

Browning (1931) writes :—

‘The behaviour of resistant strains to treatment with the corresponding drug may vary with the species of host (Breinl and Nierenstein, 1909; Mesnil and Brimont, 1908). For instance, a strain resistant to atoxyl in the mouse may not be resistant when transferred to rats, but on re-transference to mice the resistance is again apparent. It must be remembered, however, that resistance is a relative term, and other drug-fast strains have manifested their resistance also when transferred to a different species of host (Ehrlich, 1907; Mesnil and Brimont, 1908; Roehl, 1909).’

Again, later, in the same article Browning writes :—

‘That the host plays a definite part in the effect of such chemotherapeutic agents, however, would appear from the observation that a strain of trypanosomes may be resistant to a given drug in one species of host and not in another.’

While it is obvious from these quotations that each author has given prominence to the observations of Mesnil and Brimont, and of Breinl and Nierenstein, and has drawn important inferences from them, it is a curious fact that nobody has, of recent years, attempted to re-examine the matter experimentally.* This is the more

* The only reference which has come to our notice is the bald statement by Dubois (1930) to the effect that he had observed that resistance developed in the guinea-pig was fully manifest in the mouse.

surprising as the observations of the French and Liverpool writers are not in harmony with the contemporary observations of Roehl (1909) who found that a drug-fast strain of trypanosomes manifested its resistance when transferred to another species of host.

If the conclusions of Mesnil and Brimont, and of Breinl and Nierenstein, be correct, then it seems to us that it is necessary to conceive of a drug-resistant trypanosome as being resistant, not to the drug itself, or to some simple derivative of it, but to a combination of the drug and the particular serum of the animal in which the strain became drug-resistant. This is clearly what Breinl and Nierenstein (1909) had in mind when they wrote: 'We regard the resistance as an acquired immunity of the trypanosomes against the Atoxyl-serum, . . . The trypanosomes have become tolerant only to the *one* Atoxyl-serum combination, as for example in the mouse, and are still influenced by the Atoxyl-serum of the rat.'

Moreover, apart from its academic interest, the question is one of immediate practical importance. How far the development of drug-resistance determines the success or failure of treatment of trypanosomal infections of man is a problem which is now attracting much attention. Man is for obvious reasons a very unsatisfactory experimental animal, and in human trypanosomiasis the number of parasites in the peripheral blood is usually very small. These facts make it almost impossible to decide, by direct observation on man, whether or not the failure to cure in any particular case is due to development of a drug-resistant strain of parasites. The problem could probably be solved with ease by the transference of the parasites from the patient to a suitable laboratory animal, provided that such a procedure did not, in any way, modify the drug-resistance of the trypanosome strain; but if the observations of Mesnil and Brimont *et al.* be correct, this line of investigation would obviously be futile.

These considerations impressed us with the desirability of re-investigating the matter experimentally.

In our first series of experiments we used the atoxyl-resistant strain of *T. rhodesiense* to which reference has frequently been made in previous papers. This atoxyl-resistant strain was an off-shoot of a normal strain of *T. rhodesiense* which had been maintained by passage through mice since the time of isolation from

TABLE I.

Showing the development of the atoxyl-resistant strain in mice.

Mouse	Date of treatment	Dose of atoxyl mgm. per 20 gm. mouse	Result of treatment	Mouse	Date of treatment	Dose of atoxyl mgm. per 20 gm. mouse	Result of treatment
1	12.2.29	2.5	Negative 5 days	94	30.1.30	10.0	No action
	20.2.29	5.0	Not negative		31.1.30	10.0	No action
	23.2.29	10.0	Negative 8 days	95	No treatment		
2	7.3.29	5.0	No action	96	6.2.30	10.0	No action
	8.3.29	5.0	Not negative	97-106	No treatment		
	11.3.29	10.0	Negative 6 days	107	25.3.30	10.0	No action
	21.3.29	10.0	No action	108-110	No treatment		
3	28.3.29	10.0	No action	111	7.4.30	10.0	Negative 3 days
4	3.4.29	10.0	No action	112-115	No treatment		
	4.4.29	10.0	No action	116	28.4.30	10.0	No action
5	8.4.29	10.0	No action	117-118	No treatment		
6	No treatment			119	7.5.30	10.0	No action
7	13.4.29	10.0	No action		8.5.30	10.0	No action
8-28	No treatment			120-121	No treatment		
29	24.5.29	10.0	No action	122	16.5.30	10.0	No action
30-75	No treatment			123-125	No treatment		
76	11.11.29	10.0	No action	126	29.5.30	10.0	No action
77	15.11.29	15.0	No action	127	No treatment		
78-86	No treatment			128	2.6.30	10.0	No action
87	20.12.29	10.0	No action	129	6.6.30	10.0	No action
88	23.12.29	10.0	No action	130	10.6.30	10.0	No action
	24.12.29	10.0	Controlled	131	13.6.30	10.0	No action
	27.12.29	10.0	Controlled	132-137	No treatment		
	28.12.29	10.0	Negative 4 days	138	7.7.30	10.0	No action
89	6.1.30	10.0	No action	139-141	No treatment		
90	7.1.30	10.0	No action	142	15.7.30	7.5	No action
	10.1.30	10.0	No action	143-152	No treatment		
91	No treatment			153	16.8.30	10.0	No action
92	16.1.30	10.0	No action	154	19.8.30	10.0	No action
	17.1.30	15.0	No action	155-341	No treatment		
93	21.1.30	10.0	No action	342	—	—	26.3.32. Off-shoot to Rats
	22.1.30	10.0	No action				
	23.1.30	10.0	Controlled				

man in 1923. The preparation of the atoxyl-resistant variety was commenced in February, 1929, by treating infected mice with subcurative doses of atoxyl, and gradually increasing the doses as relapses occurred in the same animals and in animals of later passage. By this means a strain, which was unaffected by 10 mgm. of atoxyl per 20 gm. of mouse, was obtained within about five weeks after six doses of atoxyl. This strain has been maintained by passage through mice up to the present time. In an attempt to re-inforce the resistance, many of the mice up to Passage 154 were given a dose of atoxyl before the strain was passed into the next animal. Details are given in Table I. From Passage 155 (Aug. 1930) the strain has never been in contact with atoxyl. It is perhaps unfortunate that so many of the mice of the earlier passages were given atoxyl before the strain was passed to the next mouse, but at the time we wished, for the purpose we had in view, to be quite certain that the strain was as resistant to atoxyl as it was possible to make it; and we did not then know, what now appears to be the case, that a large proportion of these doses of atoxyl were quite unnecessary. However, the broad facts remain that the strain has been passaged through 210 mice since it was last subject to the action of any drug in August 1930; and that in mice it is still as resistant to atoxyl, and other aromatic arsenicals, as it was in the beginning; i.e., the maximum tolerated doses of atoxyl, arsacetin, tryparsamide, reduced tryparsamide, halarsol and N.A.B. have no effect on the infection. Full details regarding the behaviour of this strain, and of the normal strain from which it originated, to various arsenical and antimonial compounds are given in previous papers (Yorke and Murgatroyd, 1930; Yorke, Murgatroyd and Hawking, 1931*a*).

From Mouse 342, on 26th March, 1932, i.e., 19 months after the strain had last been subjected to the action of atoxyl during which time it had passed through 188 mice, an off-shoot was taken into rats and maintained in these animals for 11 passages comprising 27 rats. The response of the infection in these animals to various drugs was tested and compared with that of the normal strain in rats to the same drugs, with the results shown in Tables II and III.

From the data presented in these two tables, it is immediately obvious that the atoxyl-resistant mouse-strain continued to manifest very great resistance when transferred to, and passaged through, rats.

TABLE II

Result of treating rats infected with the strain of *T. rhodesiense* previously made atoxyl-resistant in mice.

Drug	Dose mgm. per 100 gm.	No. of rat passage	Degree of infection when treated Parasites per microscope field	Results of treatment		Remarks
				Time required for blood to become negative Hours	Days blood remained negative	
Atoxyl ...	37.5	1	++	No action
	50	1	++	No action
	"	11	20	No action
	"	2	10	36	2*	
	75	3	+	Infection reduced ; toxic symptoms
	"	4	10	Infection reduced ; toxic symptoms
	"	5	5	36	3	Toxic symptoms
	100	7	1	Dose lethal
	"	10	+	Dose lethal
Arsacetin...	150	1	1	Toxic symptoms
	"	4	+	Toxic symptoms
	200	1	++	36	2*	Dose lethal
	"	4	+	36	7	Toxic symptoms
Tryparsamide ...	200	3	10	No action
	"	8	10	No action
	300	8	1	No action
	"	7	++	<24	4*	
	400	8	5	Dose lethal
Red. tryparsamide thioglycollate	10	1	10	No action
	"	6	+	No action
	15	8	5	Dose lethal
Halarsol ...	3	5	+	No action
	"	3	10	No action ; toxic symptoms
	3.75	1	10	Dose lethal
N.A.B. ...	20	2	10	No action
	"	9	10	Dose lethal
	30	9	20	Dose lethal

* Rat died without relapse.

TABLE III

Result of treating rats infected with the normal strain of *T. rhodesiense* previously passed through mice.

Drug	Dose mgm. per 100 gm.	No. of rat passage	Degree of infection when treated Parasites per microscope field	Results of treatment		Remarks
				Time required for blood to become negative Hours	Days blood remained negative	
Atoxyl ...	3.75	2	5	No action
	"	3	5	No action
	"	3	5	36	1	
	5	1	1	36	4	
	"	1	1	36	3	
	7.5	1	10	36	3	
	"	1	+	48	3	
	"	1	10	24	4	
	10	1	+	<18	15	
	"	1	+	<18	15	
	15	5	1	12	6	
	"	1	+	12	11	
	"	1	+	12	25	
	25	10	15	12	11	
	"	7	20	12	Cured	
Arsacetin...	5	1	1	No action
	10	1	1	24	4	
	15	2	5	<24	5	
	20	2	3	<24	9	
Tryparsamide ...	15	1	5	<18	3*	No action
	"	5	20	
	20	2	5	36	2	
	"	5	5	36	4	
Red. tryparsamide thioglycollate	30	2	2	<24	9	
	0.05	3	10	No action
	0.125	1	10	<18	2	
	"	4	+	<18	3	
	0.25	1	10	<18	2	
Halarsol ...	"	4	+	<18	5	
	0.5	3	5	<18	6	
	0.05	7	20	<18	1	
	0.1	1	5	<18	2	
N.A.B. ...	"	8	20	<18	11	
	0.2	1	10	<18	7	
	0.3125	1	3	No action
	0.625	1	+	<18	6	
	"	1	3	<18	5	
	1.0	7	+	<24	4	

* Rat died without relapse.

It is true that occasionally with exceedingly large doses (bordering on the toxic) of certain drugs we did succeed in causing a transient disappearance of parasites from the peripheral blood of rats infected with the resistant strain; but reference to our earlier papers will show that this was likewise the case when mice, infected with the same strain, were treated with corresponding doses of the same drugs. For the sake of comparison we have recorded in Table IV the approximate minimum effective doses* of the various drugs for both the normal and the atoxyl-resistant strains in mice (Yorke, Murgatroyd and Hawking 1931a), and for the same strains after transference to rats.

TABLE IV

Comparison of minimum effective doses of various drugs for the normal and atoxyl-resistant strains in mice and for the same strains transferred to rats.

Drug	Infections in mouse			Infections in rat		
	M.E.D. mgm. per 20 gm. mouse		Resistant factor*	M.E.D. mgm. per 20 gm. rat		Resistant factor*
	Normal strain	Resistant strain		Normal strain	Resistant strain	
Atoxyl	2	15+	7.5+	1	15+	15+
Arsacetin	4	40+	10+	2	40	20
Tryparsamide	6	100+	16+	4	60+	15+
Red. tryparsamide thio- glycollate	0.04	2.5++	60++	0.025	2++	80++
Halarsol	0.02	0.75++	37.5++	0.01	0.6++	60++
N.A.B.	0.125	4+	32+	0.125	4++	32++

$$\text{*Resistant factor} = \frac{\text{M.E.D. for resistant infection.}}{\text{M.E.D. for normal infection.}}$$

+ and ++. In the columns for the M.E.D. of the resistant strain 15+ signifies that this dose reduces the infection but is not sufficient to sterilize the blood; 2.5++ signifies that this dose exerts no influence on the infection. In the columns for the resistant factors 7.5+ signifies that it is probably not much greater than 7.5, and 60++ signifies that it is probably considerably greater than 60.

It will be seen from this Table that, although in the case of the normal strain, the M.E.D. is rather lower in rats than in mice, the resistant strain withstands the maximum tolerated doses in both

* The minimum effective dose (M.E.D.) is the smallest dose which suffices to clear the blood of the majority of the treated animals, irrespective of time.

animals, and the resistant factors $\frac{\text{M.E.D. for resistant infections}}{\text{M.E.D. for normal infections}}$ are just as great in rats as they are in mice. From this it seems clear that the mouse atoxyl-resistant strain manifested the same degree of resistance after transference to rats as it exhibited in its original host.

Without going into details it may be recorded that after the 10th rat passage had been reached both strains were returned to mice and their resistance to various drugs examined. Their response was exactly the same as it was before their passage through rats.

In the second series of experiments we employed a tryparsamide-resistant strain of *T. rhodesiense*. This was prepared in the usual way by treating infected mice with gradually increasing doses of the drug. Details are given in Table V.

TABLE V

Showing the development of the tryparsamide-resistant strain in mice.

Mouse	Date of treatment	Dose of tryparsamide, mgm. per 20 gm. mouse	Result of treatment
1	5.2.31	6	Negative 6 days
	11.2.31	9	Negative 10 days
	21.2.31	12	Negative 9 days
2	7.3.31	24	Not negative
	9.3.31	36	No action
3	11.3.31	36	No action
4	13.3.31	72	No action
	14.3.31	48	No action
5	17.3.31	96	No action
	18.3.31	48	No action
6	No treatment		
7	24.3.31	108	Negative 6 days
8-30	No treatment		
31	20.6.31. Off-shoot to rabbits.

On 20th June, 1931, three months after the strain had last been in contact with tryparsamide, during which period it had been passaged through 24 mice, an off-shoot was taken into rabbits and maintained in these animals for 16 passages.

The response of the infection to tryparsamide was tested in a number of these rabbits as is shown in Table VI from which it is seen that the largest doses of the drug which could be tolerated had not the slightest effect on the infection; i.e., if signs of disease were present when treatment began there was no amelioration of the condition, and if treatment was given early before signs had developed the disease progressed with the same rapidity and severity as in the control untreated animals. These results are in striking contrast with those obtained in rabbits infected with the normal strain in which relatively small doses of tryparsamide e.g., 0.25 gm., per kilo of body weight, invariably cleared the blood and produced pronounced improvement.

When the resistant strain was returned to mice after sixteen passages in rabbits, it was found to be unchanged and just as resistant to various drugs as it was originally.

In the third series of experiments *T. rhodesiense* was made resistant to tryparsamide in rabbits by the usual procedure. When the resistance was such that maximum tolerated doses of the drug had no effect on the infection the strain was transferred to mice. In these animals likewise it proved to be completely resistant to the aromatic arsenicals mentioned in Table IV.

In addition to these *in vivo* experiments, we have at our disposal the results of a large number of *in vitro* experiments which appear to us to have a direct bearing on the subject under consideration.

These experiments fall into two groups. In the first, the resistance *in vitro* to an actively trypanocidal arsenical (reduced tryparsamide) of the normal and atoxyl-resistant strains obtained from mice was compared with that of the same strains after passage through a number of rats. The citrated heart blood of the heavily infected mouse or rat was centrifuged at low speed in order to throw down the red corpuscles. The supernatant fluid was then centrifuged at high speed and the deposited trypanosomes washed several times in large volumes of nutrient medium (i.e., rabbit-serum + Ringer-glucose solution, equal parts). Finally a suitable suspension of the washed

TABLE VI

Showing the effect of treatment with tryparsamide of rabbits infected with the strain of *T. rhodesiense* previously made tryparsamide-resistant in mice.

Rabbit Passage	Day of commencement of treatment	Symptoms at commencement of treatment	Treatment	Result of treatment	Remarks
			Dose in gm./kg.		
1	23rd	Present	0.75 on 23rd day 0.5 on 24th day 1.0 on 28th, 31st, 34th and 37th days	No amelioration	Died on 38th day with marked signs of disease and with trypanosomes in blood.
2	10th	Absent	1.0 on 10th and 14th days 0.75 on 16th day 1.0 on 19th day	No action	Died on 24th day, with marked signs of disease and trypanosomes in blood.
3	10th	Absent	1.0 on 10th day	No action	Disease developed in usual manner.
4	4th	Absent	1.0 on 4th, 7th, 11th, 14th and 18th days	No action	Trypanosomes persisted in blood, and disease developed in usual manner.
9	18th	Present	0.8 on 18th day	No action	Trypanosomes persisted in blood and animal died on 22nd day.
15	9th	Absent	0.8 on 9th, 12th, 16th and 20th days	No action	Trypanosomes persisted in blood, and disease developed in usual manner.
16	17th	Present	0.8 on 17th, 20th, and 24th days	No amelioration	Trypanosomes persisted in blood, and disease developed in usual manner.

TABLE VII

Showing the minimum concentration of reduced tryparsamide thioglycollate which destroyed, *in vitro*, at 37°C., the normal and atoxyl-resistant strains of *T. rhodesiense* obtained from the mouse, and also from the rat. The nutrient medium consisted of rabbit serum and Ringer-glucose solution in equal parts.

Experiment	Immediate origin of strain	Minimum lethal concentration of reduced tryparsamide thioglycollate			
		Normal strain		Atoxyl-resistant strain	
		Within 6 hours	Within 24 hours	Within 6 hours	Within 24 hours
I	From mouse ...	1 : 25,600,000	1 : 102,400,000	1 : 100,000	1 : 800,000
	From rat (passage 1)	1 : 25,600,000	1 : 102,400,000	1 : 100,000 to 1 : 200,000	1 : 800,000
II	From mouse ...	1 : 12,800,000	1 : 102,400,000	1 : 50,000	1 : 400,000
	From rat (passage 6)	1 : 12,800,000 to 1 : 25,600,000	1 : 102,400,000	1 : 50,000	1 : 400,000

trypanosomes was made in nutrient medium, and equal volumes (0.025 c.c.) added to a series of tubes each containing 0.5 c.c. of nutrient medium in which were dissolved various quantities of reduced tryparsamide thioglycollate.

It will be seen from Table VII that the results of experiments of this nature were the same whether the trypanosomes were obtained direct from mice, or after transference of the strains to rats. In each case the normal strain was destroyed by a concentration of about 1 : 102,400,000 of reduced tryparsamide within a period of 24 hours, whereas the resistant strains required a concentration of about 1 : 800,000 to 1 : 400,000.

The second group of experiments was designed with the object of ascertaining whether the species of animal supplying the serum for the nutrient medium in any way affected the result. The general procedure was essentially the same as that described for the previous experiment, except, firstly, that the trypanosomes were always obtained from mice—in all, eight strains were used, made resistant in mice to atoxyl, tryparsamide, arsacetin, reduced tryparsamide, halarsol, N.A.B., stibenyl and acriflavine respectively—and secondly, the serum used for the nutrient medium came alternately from rabbit and from sheep. The results of experiments in which the medium was made from rabbit serum were the same as those in which it was made from sheep serum ; and it might be added it was immaterial whether the serum was deactivated or not. In all experiments the resistant strains withstood much greater concentrations of the drug than did the normal strain.

These *in vitro* experiments seem to us to afford convincing evidence that drug-resistance is a character inherent in the trypanosomes themselves ; it is not influenced by transference of the strain to other species of host, and it is equally manifest whether the drug is encountered in the presence of rabbit serum or of sheep serum.

From this work we have therefore failed to obtain any evidence that a strain of trypanosomes, made resistant to an aromatic arsenical compound in one species of animal, evinced the slightest loss of resistance when transferred to another species of animal. A strain, made resistant to atoxyl in the mouse, continued to manifest its full resistance when transferred to, and passaged through, rats, as also did a strain, made resistant to tryparsamide in the mouse, when

transferred to and passaged through rabbits; similarly a strain, made resistant to tryparsamide in the rabbit, manifested complete resistance when subsequently transferred to mice. These observations are so markedly at variance with the statements made by Mesnil and Brimont, and by Breinl and Nierenstein, that we have thought it necessary to examine critically the papers of these writers and to consider whether their experimental records warranted the inferences which they, and those by whom they are quoted, have drawn from them.

Briefly, Mesnil and Brimont's experiments were as follows:—

A horse, experimentally infected with *T. evansi* from Mauritius, was treated with atoxyl; it received 34 gm. of the drug within a period of 28 days. The resistance of the parasites is said to have increased during the course of treatment, because a condition of affairs was reached when ordinary doses of the drug had no action, but larger doses were effective in causing parasites to disappear from the blood. A mouse was then inoculated from the horse and developed an infection upon which an ordinary dose of atoxyl had no action. A second mouse was subinoculated from the first and given a dose of atoxyl, after which the strain was passed into a third mouse which was likewise given a dose of atoxyl. A fourth mouse was then subinoculated and from this point the strain was passaged through a series of 90 mice during a period of nearly a year. None of these mice were treated before the strain was passed into the next animal except the 5th mouse of the series, which had a dose of arsenious acid. This strain Mesnil and Brimont designate *Strain R*. *Strain J* is the same as the preceding strain except that it originated from the 6th mouse of *Strain R* after it had been treated with atoxyl. The drug is stated to have had a feeble action in this mouse prolonging life for a few days, but not completely clearing the blood of trypanosomes. *Strain J* was passed through 140 untreated mice during a period of nearly 18 months. *Strain J₁* originated from *Strain J* at its 68th passage. With the object of re-inforcing the resistance, Mice 1, 2, 4 and 5 of the new series were given atoxyl before the strain was passed on into the next mouse. *Strain J₁* was maintained for 7 months during which period it was passed through 67 mice.

Many of the mice of each series were tested with the maximum tolerated dose of atoxyl (amount not stated). In the case of *Strain R* and *Strain J* it was found that whilst in many mice the infection was completely resistant, in others it was to some extent controlled, in that life was prolonged for 1 to 3 days; and in a few mice trypanosomes actually disappeared temporarily from the circulation, and life was prolonged for 6 to 10 days. In the case of *Strain J₁* it is recorded that the action of the drug was always nil, or practically nil, the greatest prolongation of life being 4 days.

It is further remarked that there was no evidence that the various strains became more sensitive to atoxyl as time went on. The individual variations in susceptibility to the drug were not to be correlated with the length of time which had elapsed since the strains were made resistant to atoxyl, but they were dependent on 'particularités individuelles des souris infectées.'

These observations suggested to Mesnil and Brimont that drug-resistance on the part of trypanosomes required for its manifestation a certain participation of the host; and this consideration led them to inquire whether transference of the strains to a different vertebrate host in any way affected their resistance to atoxyl.

From Mouse J 67 a rat was inoculated; this was cured by a dose of atoxyl equalling 25 mgm. per 100 gm. From Mouse J 68 two rats and a control mouse were inoculated; the rats were treated with 24 mgm. of atoxyl per 100 gm., and the trypanosomes disappeared from the blood within twenty-four hours, whereas in the case of the mouse, the infection was uninfluenced by a dose of 4.7 mgm. per 20 gm. of body weight.

From mice infected with the reinforced resistant *Strain J₁* a number of rats were inoculated and treated with atoxyl in doses equalling 20 mgm. per 100 gm. These doses always caused the disappearance of trypanosomes from the blood, sometimes after an interval of more than twenty-four hours, but relapses within three to six days were frequent. The authors infer from this that a certain resistance of the strain was manifest in rats. The strain was carried through a series of 43 rats during a period of 157 days, and it is recorded that the resistance did not alter but remained feebly manifest throughout.

The following examples are given in support of these contentions. Two rats of the 41st passage were given respectively 17.5 mgm. and 25.0 mgm. of atoxyl per 100 gm.; trypanosomes disappeared from the peripheral blood in forty-eight hours and did not reappear for several days. In contrast, two mice were inoculated from Rat 40; the first was given 3.1 mgm. of atoxyl per 20 gm. of body weight without any influence on the infection, the second was given 3.6 mgm. of atoxyl per 20 gm. and life was prolonged for forty-eight hours. It is noted that mice infected with the normal strain of surra from India were cured by 3.9 mgm. of atoxyl per 20 gm.

Mesnil and Brimont also record a few observations made with other hosts. From Mouse J₁ 9 a dog was infected, and treated with 10 mgm. of atoxyl per kilo. of weight; the treatment was without effect. From Mouse J₁ 15 another dog was infected, and treated with two similar doses of atoxyl, also without effect. The authors admit that this dose (1 mgm. per 100 gm.) is relatively feeble, but state that it could not be exceeded on account of the great susceptibility of dogs to the drug.

Somewhat similar observations were made with guinea-pigs. Four of these animals infected with *Strain J₁* were treated with doses of atoxyl varying from 3.3 mgm. to 3.75 mgm. per 100 gm.; trypanosomes disappeared only after an interval of forty-eight hours, and relapses occurred. It is remarked that similar doses of atoxyl given to guinea-pigs infected with the normal Indian strain of surra caused trypanosomes to disappear from the blood in less than twenty-four hours, and that relapses were long delayed.

Before considering the interpretation which Mesnil and Brimont themselves have drawn from these observations, we might at this point briefly refer to the somewhat similar work carried out about the same time in Liverpool by Moore, Nierenstein and Todd (1908), and by Breinl and Nierenstein (1908).

Moore, Nierenstein and Todd give brief, and quite inadequate, records of a few similar observations. From the first experiments, which were made with an atoxyl-fast strain of *T. brucei* in mice, received from Ehrlich, it is concluded that the strain was atoxyl-resistant in three mice but not in two rats and a dog; and from the second, made with an atoxyl-fast strain of *T. brucei* in rats, prepared by themselves, it is concluded that the strain was atoxyl-fast in three rats but not in two mice and a dog.

Breinl and Nierenstein (1908) state that they made a strain of *T. brucei* in a donkey resistant to atoxyl within a period of three months by twice-weekly injections of 1 gm.

of the drug. The strain was then transferred to rats. The rat inoculated from the donkey was given two injections of 0.5 c.c. of a 5 per cent. solution of atoxyl (25 mgm. each injection), and trypanosomes disappeared from the blood on the third day after the commencement of treatment; whereas, in control rats infected with the normal strain, parasites disappeared from the blood in six to nineteen hours after a single injection of the drug. The second passage of the donkey-strain in rats behaved exactly as did the normal strain. According to Breinl and Nierenstein, these experiments show that when a strain of *T. brucei*, made resistant to atoxyl in a donkey, is transferred to rats it exhibits but a slight and transient resistance in the first passage only. The strain was maintained in the laboratory by fourteen passages through rabbits, guinea-pigs and rats during a period of six months. At most it exhibited only the slightest atoxyl-resistance in these animals. It is recorded that when, at the end of this time, the strain was returned to a donkey it exhibited its pristine atoxyl-resistance.

The general conclusion drawn by Breinl and Nierenstein from their experiments is that atoxyl-resistance on the part of a trypanosome strain holds only for the species of animal in which it was established, and disappears when the strain is transferred to another species of animal, to be restored once more when it is returned to the original host. Mesnil and Brimont were unable to agree entirely with this view because their atoxyl-resistant strain from the horse proved resistant also when transferred to mice. Nevertheless they add: "Nos expériences comparées chez le rat et la souris prouvent en plus que le milieu-hôte a une grande importance; pour être exact, il faut dire que la race est résistante *dans un organisme donné*."

We now propose to pass to a critical examination of these observations and to consider how far, in the light of present knowledge, they warrant the fundamental conclusions which have been drawn from them; but before doing so it is desirable to draw attention to two points regarding drug-resistance, and the means whereby it can be assessed, which have emerged as the result of recent work.

A. In all work of this kind it is essential to bear in mind a fact which is frequently overlooked, viz.:—*Resistance is a relative term*. We have found that it is quite easy to produce from a normal strain of trypanosomes a whole series of strains of ever increasing resistance until finally a strain is obtained upon which doses of the aromatic arsenicals, representing the maximum amount tolerated by the vertebrate host, have not the slightest effect. This fact is clearly illustrated in one of our previous papers (1931, b).

B. Whilst atoxyl is an excellent drug for the production of such strains, it is an unsatisfactory reagent with which to examine for

different degrees of resistance. For this purpose it has the following disadvantages :—

1. Atoxyl is a pentavalent arsenical compound, and as such is inert, and must be reduced in the body of the vertebrate host to its corresponding arsenoxide before it can exert a trypanocidal action. There is thus an interval between its administration and the commencement of its action, during which a considerable amount of the drug is excreted. Consequently the concentration of *p*-aminophenylarsenoxide available as a trypanocide, after the administration of a given dose of atoxyl, will depend in some measure on the rate of excretion of atoxyl and on its rate of reduction in the vertebrate host. There is a considerable mass of evidence that these factors vary not only in animals of different species, but also in individuals of the same species.
2. Atoxyl has a relatively small therapeutic range, i.e., in the case of mice infected with our normal strain of *T. rhodesiense* there is little difference between the maximum tolerated dose (6—8 mgm. per 20 gm.), the minimum curative dose (6 mgm.) and the minimum effective dose (2 mgm.).
3. Atoxyl has a relatively small resistant factor, i.e., even strains, such as our atoxyl-resistant strain, which have attained to the maximum degree of resistance to the aromatic arsenicals, withstand *in vitro* only four to eight times the maximum concentration of *p*-aminophenylarsenoxide withstood by the normal strain; and consequently mice infected with such highly resistant strains can sometimes be sterilized temporarily by sublethal doses of atoxyl, e.g., 10—15 mgm. per 20 gm. mouse (Yorke & Murgatroyd, 1930, and Yorke, Murgatroyd and Hawking, 1931, a).

For the determination of varying degrees of resistance to the aromatic arsenicals we have found such trivalent compounds as reduced tryparsamide thioglycollate, or halarsol, to be immeasurably superior to atoxyl, for the following reasons :—

1. Reduced tryparsamide and halarsol are, as such, actively trypanocidal, and their action commences immediately they are administered. These facts eliminate one of the variables dependent on the host and lessen the effect of the other.

2. Reduced tryparsamide and halarsol have a relatively wide therapeutic range, e.g., in mice infected with our normal strain of *T. rhodesiense*, the maximum tolerated dose of reduced tryparsamide is 2.5 mgm. per 20 gm. of mouse, the minimum curative dose is 0.5 mgm. and the minimum effective dose is 0.04 mgm. : the M.T.D. is thus about sixty times the M.E.D. The range in the case of halarsol is equally great.
3. Reduced tryparsamide and halarsol have relatively large resistant factors, e.g., strains such as our atoxyl-resistant strain which have attained to the maximum degree of resistance to the aromatic arsenicals,* withstand *in vitro* about 250 times the concentration of reduced tryparsamide thioglycollate withstood by the normal strain; and consequently even a sub-lethal dose of 2.5 mgm. (60 times the M.E.D. for the normal strain) never has the slightest effect in mice infected with the resistant strain.

Prolonged experience has shown us that with reduced tryparsamide thioglycollate and halarsol it is possible to determine the precise degree of resistance to aromatic arsenicals possessed by any particular strain of trypanosomes with much greater accuracy than with atoxyl.

It seems to us that these facts must be kept clearly in mind when examining the experimental observations presented by Breinl and Nierenstein, and by Mesnil and Brimont. The experimental data contained in the paper of Moore, Nierenstein and Todd are so scanty, and so much essential information, e.g., the weight of the animals used and consequently the exact dosage given, is omitted, that it seems impossible to draw any inference from them. This view is supported by a passage which occurs in a later paper by Breinl and Nierenstein (1909) which runs as follows :—" In further experiments, however, with an Atoxyl-resistant strain in mice, sent to us by Professor Ehrlich, we observed that the resistance after sub-inoculation from mice into rats was still well marked in the latter animals even after three passages. This result, also obtained by Roehl, seemingly contradicts our previous observations that the

* In previous papers (1930 and 1931a) we have shown that an atoxyl-fast strain is resistant to all the commonly employed aromatic arsenicals; and in later, still unpublished, work we have found that strains made resistant to any of these aromatic arsenicals are likewise resistant to all the others. It follows therefore that any one of the aromatic arsenicals, e.g., reduced tryparsamide, can be used to assess the degree of resistance in the case of any of these strains.

resistance is confined to one animal species *only*." As, presumably, this is the strain used by Roehl (1909) in the work from which he concluded that atoxyl-resistance was still manifest when the parasite was transferred to rats, it seems safe to conclude that Moore, Nierenstein and Todd had no sound experimental evidence to support their contention.

Nor are the experiments recorded shortly afterwards by Breinl and Nierenstein any more convincing. In the first place, they bring forward no satisfactory proof that the strain which was transferred from their donkey to rats had attained to any substantial degree of atoxyl-resistance, beyond the general statement that in 26 infected donkeys used by them, atoxyl-resistance was generally obtained as the result of twice-weekly injections of 1 gm. of atoxyl for a period of three and a half months. Certainly the degree of atoxyl-resistance was not very great, as we are informed that, when the strain was returned to a donkey after its passage through fourteen laboratory animals, although 1 gm. of atoxyl failed to sterilize the blood, a dose of 2 gm. did so. In the second place, the evidence that the strain, when transferred from the donkey to rats, manifested a slight degree of atoxyl-resistance in the first rat, and none in the second, is most unsatisfactory. In the absence of information regarding the weights of these two rats, we cannot even be sure that they had similar doses of the drug. Then again, when referring to the strain during its fourteen passages through rabbits, guinea-pigs and rats the authors write: 'Bemerkenswert ist, dass während dieser Zeit keine, auch nur die geringste Atoxylfestigkeit in diesen Tieren festgestellt werden konnte.' This sentence to our minds is the explanation of the whole matter. The strain was but slightly atoxyl-resistant when transferred from the original donkey, and it remained so during its passage through the various laboratory animals; the authors' technique proved insufficiently delicate to recognize this fact, especially as they apparently ignored such important considerations as the administration of exactly comparable dosage based on the weight of the animals, and the possibility that the rate of excretion of the drug might vary in animals of different species and even in individuals of the same species.

Again, it is interesting to note that Breinl and Nierenstein misquote Mesnil and Brimont when they write that the latter found that a strain of *T. evansi*, which they had made atoxyl-resistant in

the horse, no longer manifested this resistance when transferred to mice. This is certainly not Mesnil and Brimont's interpretation of their own experiments. These authors definitely state that the strain was atoxyl-resistant in the mouse, and, as a matter of fact, all their subsequent work was based on this assumption.

Whilst Mesnil and Brimont produce very convincing evidence that their horse-strain manifested a certain degree of resistance in mice, it is quite clear that the resistance was far from complete. We base this opinion on the statement of Mesnil and Brimont that in some of the mice infected with *Strains R* and *J* life was prolonged for several days, and in others the blood was actually temporarily sterilized, and life prolonged for 6 to 10 days, after the administration of the maximum tolerated dose of atoxyl. Unfortunately, the amount of the maximum tolerated dose is not recorded, but from various statements made in this, and other papers (1908, a), we gather that it was 4 mgm. to 6 mgm. per 20 gm. mouse. This omission is the more unfortunate as we have no information regarding the action of atoxyl in mice infected with the normal strain, although there is a statement to the effect that 4 mgm. of atoxyl per 20 gm. mouse cured mice infected with *T. evansi* of India. From this information, such as it is, we infer that *Strains R* and *J* were but slightly atoxyl-resistant, and that the maximum tolerated dose of atoxyl for the mouse was almost the minimum effective dose for these strains. This, in our opinion, affords a reasonable explanation of the fact that the blood of certain mice was cleared by the maximum tolerated dose, whereas in other mice the same dose had little or no action. The individual differences are to be explained on variations in the rates of excretion and of reduction of the drug in the different mice. The very few experiments recorded by Mesnil and Brimont concerning the results of treating rats infected with *Strain J* can equally well be explained on these grounds.

When we turn to the experiments performed with the reinforced *Strain J₁*, we find evidence that it was definitely more resistant than were the other two strains, in that it is recorded that the action of the drug was always nil, or practically nil, the greatest prolongation of life being four days. On the other hand, consideration of the experiments relating to the treatment of rats infected with this strain shows that it manifested in rats a definitely greater degree of resistance than did *Strain J*; and, indeed, this fact is admitted by

Mesnil and Brimont when they state that, during the passage of *Strain J₁* through a series of 43 rats, its resistance was feebly manifest throughout.

Mesnil and Brimont's experiments relating to dogs and guinea-pigs require no comment beyond the statement that they are obviously inadequate to warrant any conclusion.

To summarize our criticism of the work of Breinl and Nierenstein, and of Mesnil and Brimont, we consider that these authors failed to produce any evidence warranting the conclusions reached by them. The main desiderata which led to their mistaken inferences were :—

1. Failure to keep prominently in mind the fact that *resistance is relative*.

2. The use of strains which were of a comparatively slight degree of resistance. This fact escaped the authors' attention : partly because they omitted to determine exactly the effect of atoxyl on infections of the parent non-resistant strain in mice and rats respectively ; and partly because they employed, as a measure of resistance, a drug (atoxyl), which, for reasons we have mentioned, is ill-adapted for the purpose.

3. Failure to interpret correctly the fact that the same dose of atoxyl has by no means invariably the same effect on different mice infected with the same strain. In our opinion, the true explanation of this phenomenon, which indeed is clearly brought out in Mesnil and Brimont's own work, is that the rates of excretion, and of reduction, of atoxyl vary considerably in different mice, and consequently the concentration of *p*-aminophenylarsenoxide, available as a trypanocide, likewise varies considerably in individual mice. These individual variations apply equally in the case of other animals ; and they assume an enhanced significance when it is a matter of comparing the action of atoxyl in infected mice with its action in infected rats. As reference to Table IV of the present paper shows, the minimum effective dose of atoxyl per 20 gm. of body weight is less for rats infected with the normal strain of *T. rhodesiense* than it is for mice infected with the same strain : this applies also in the case of each of the other arsenicals examined. Consequently, if the same dose per 20 gm. of body weight be given to mice and rats infected with the same strain, we should naturally expect a greater effect to be produced in the latter animals. If the

drug be given for the treatment of infections produced by a strain which is relatively but slightly resistant, then it is quite possible that the same dose per 20 gm. of body weight might suffice to sterilize the blood of the rat although it was insufficient to sterilize that of the mouse.

The results of our own experiments are not confused by issues of this sort: firstly, because we employed strains which had attained to the maximum degree of resistance; and secondly, because we took the precaution of comparing the M.E.D. for the normal strain in mice with that for the resistant strain in the same animal, and of comparing the M.E.D. for the normal strain in rats with that of the resistant strain in these animals. When this was done, it was found that the fraction $\frac{\text{M.E.D. for the resistant strain}}{\text{M.E.D. for the normal strain}}$ was the same for both animals.

In conclusion, therefore, we believe that this inquiry has shown definitely that resistance to the aromatic arsenicals is a stable character which is inherent in the trypanosomes themselves and is in no way dependent upon, or modified by, the particular vertebrate host in which the parasite happens to find itself. The trypanosome is resistant to the drug itself, or, in the case of a pentavalent arsenical, to its corresponding trivalent derivative. We have found no evidence to support Breinl and Nierenstein's hypothesis that the resistance is directed against a combination of the drug and of the specific serum of the host in which the strain became resistant.

SUMMARY

1. Reference is made to the observations of Mesnil and Brimont, and of Breinl and Nierenstein and others, to the effect that a strain of trypanosomes made resistant to atoxyl in the mouse may lose this resistance when transferred to other hosts.

2. Study of recent reviews and articles relating to chemotherapy indicates that these observations have not only been accepted without question, but have had an important influence on modern conceptions of chemotherapeutic processes and of drug-resistance. Moreover, apart from its academic interest, the matter is one of immediate practical importance in so far as it touches the investigation of the problem whether the development of drug-resistance is a cause of

the failures so frequently encountered in the treatment of human trypanosomiasis. For obvious reasons, it is practically impossible to investigate the problem by direct observations on man himself; and, if resistance is lost on the transference of the trypanosome from man to laboratory animals, the fact constitutes a most serious obstacle to the elucidation of this fundamental question. These observations impressed upon us the necessity for re-investigating the whole subject.

3. Our experiments failed to confirm the observations of Mesnil and Brimont, and of Breinl and Nierenstein and others. A strain of *T. rhodesiense*, made resistant to atoxyl in the mouse, continued to manifest its full resistance when transferred to, and passaged through, rats; as also did a strain made resistant to tryparsamide in mice when transferred to, and passaged through, rabbits; similarly a strain made resistant to tryparsamide in the rabbit manifested complete resistance when subsequently transferred to mice. Moreover, *in vitro* experiments likewise yielded results which indicate that the character of drug-resistance is not modified by transference of the trypanosome from one host to another.

4. Attention is drawn to the fact that resistance is a relative term and that all degrees of resistance, varying from the sensitiveness of the normal strain to a condition of complete insensitiveness to the largest doses which can be tolerated by the host, can easily be produced. Whilst atoxyl and other pentavalent arsenicals are excellent drugs for the production of such strains they are, for reasons stated, relatively poor indicators of the degree of resistance attained by any strain. The trivalent arsenicals, halarsol and reduced tryparsamide thioglycollate, are immeasurably superior in this respect, and enable the degree of resistance to aromatic arsenicals, attained by a strain of trypanosomes, to be assessed with great accuracy and within wide limits.

5. We have subjected the papers of Mesnil and Brimont, and of Breinl and Nierenstein to critical examination, and, for reasons stated, have reached the opinion that the data supplied therein warranted neither the sweeping generalization of Breinl and Nierenstein 'dass die erworbene Atoxylfestigkeit nur für die betreffende Tierspezies gut hält, in welcher die Atoxylfestigkeit erworben wurde,' nor even the more modest conclusion of Mesnil

and Brimont : 'Nos expériences comparées chez le rat et la souris prouvent en plus que le milieu-hôte a une grande importance ; pour être exact, il faut dire que la race est résistante *dans un organisme donné*.'

6. Our investigations convince us that resistance to the aromatic arsenicals is a stable character which is inherent in the trypanosomes themselves, and is in no way dependent upon, or modified by, the particular vertebrate host in which the parasite happens to find itself. The trypanosome is resistant to the drug itself, or, in the case of a pentavalent arsenical, to its corresponding trivalent derivative. We have found no evidence to support Breinl and Nierenstein's hypothesis that the resistance is directed against a combination of the drug and of the specific serum of the host in which the strain became resistant.

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NATURAL MALARIA INFECTION OF HOUSE-FREQUENTING *ANOPHELES* MOSQUITOES IN UGANDA

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I. INTRODUCTION

The main part of this inquiry, the infectivity survey of the *Anopheles* of Kampala and Jinja, was carried out during my secondment to the Uganda Malaria Survey Unit, which was inaugurated in May, 1929, by Colonel S. P. James, Adviser on Tropical Diseases to the Ministry of Health, while on a visit to East Africa, undertaken at the request of the Secretary of State for the Colonies to advise on anti-malarial measures. The work was carried out under the direction of Dr. H. Lyndhurst Duke, O.B.E., formerly Deputy Director of Laboratory Services and now Director of the Human Trypanosomiasis Institute, Entebbe. On my return from leave in June, 1931, I was seconded to the Entomological

Section of the Department of Agriculture, and continued the study at Fort Portal and later at Mbale.

The four regions in which this inquiry has been conducted all differ considerably in their chief physical features. Kampala is an inland town situated on hillsides with intervening swamps and artificially-made breeding places; Jinja is at the lakeside, which is fringed by tall reeds and papyrus swamp, with the source of the River Nile near by; Fort Portal is a town of high elevation, confined between extensive swamps of quite a different flora from those at lower altitudes; whereas Mbale lies below Mt. Elgon on the edge of the Bugwere plains betwixt two mountain streams. It may therefore be inferred that the six species of *Anopheles* found to be concerned in the dissemination of malaria are fairly representative of the carriers of this disease in Uganda. The principal vectors were found to be *Anopheles costalis* (Giles) (*gambiae* Giles) and *Anopheles funestus* Giles. These two species were present in extremely large numbers in native habitations about Kampala, and during the year's survey of this township the opportunity was taken of dissecting as many as time would permit, in order to obtain the fullest information on their seasonal behaviour and incidence of infection. In the case of the other species of *Anopheles*, particularly where the record of malaria infection was a new one or the species rare, a special effort was made to obtain as many specimens as possible, and collections were not strictly confined to those huts under regular observation.

I take this opportunity to express my grateful thanks to Colonel S. P. James, under whose guidance this inquiry was commenced; to my former chief, Dr. H. L. Duke, O.B.E., for his friendly advice and constant support; and to Sir Guy A. K. Marshall, C.M.G., for his kindly interest in the study while on a visit to Uganda. I am particularly indebted to Mr. W. G. Adams, Provincial Commissioner, Dr. S. Forrest, Senior Medical Officer, Dr. R. S. McElroy, Health Officer, and Mr. G. L. R. Hancock, Assistant Entomologist, for their help in various ways. I specially wish to thank Dr. N. C. Macleod, Malaria Officer who was in charge at Kampala, for allowing me to devote so large a part of my time to this study; Mr. H. Hargreaves, Government Entomologist, and Mr. G. H. E. Hopkins, Entomologist, for facilitating the investigation at a later

date ; and Mr. T. W. Chorley, for his assistance at Jinja. Finally I must acknowledge the services of my native laboratory attendant, Eria Kagwa, who, with the most painstaking care, performed almost all the dissections for my examination.

II. METHOD OF KEEPING *ANOPHELES* AND ROUTINE PROCEDURE

To simplify the diagnosis of the oöcyst on the stomach wall and to allow for the alimentary tract to become freed of blood, the female *Anopheles* were kept for one week before dissection. By this time, it was found, the oöcyst had developed to a stage easily discernible under the 1/6 inch lens of the microscope. With this object in view it was necessary to use a convenient system whereby 300 to 400 *Anopheles* could be kept alive in captivity. The method of housing together a number of mosquitoes in jars proved unsuitable to this investigation. The procedure adopted was to give each mosquito a temporary home of its own by employing 6 by $\frac{3}{4}$ inch test tubes provided with a wad of plain absorbent lint at the bottom, which, as the mosquito is liable to catch its legs in the soft hairy surface, was arranged with the woolly side downwards. The insect was imprisoned by covering the mouth of the tube with a piece of cotton netting secured by a rubber band. Sterile water was then added, in sufficient quantity to soak the lint, by holding the tube at an angle and allowing the water to run slowly down the side from a pipette held firmly against the netting. In this manner damage to the mosquito was avoided. A raisin was placed upon the netting, and the tubes were set upright in racks in a cool, well-ventilated room of the laboratory. Under these conditions there were comparatively few deaths. The insect laid its eggs, while any mosquito that died fell on to the damp lint and remained moist, pending dissection. Prior to dissection, the species of each *Anopheles* was determined from the dead specimen. Dissections of the alimentary tract and salivary glands were carried out in normal saline and examined in the fresh state under the 1/6 objective. To render oöcysts on the under surface visible, the wrinkled stomach was depressed by cautiously removing with filter paper some of the fluid from beneath the coverslip ; the same degree of compression

was aimed at each time, but rupture of the stomach was avoided as far as possible. After a preliminary examination of the salivary glands intact, the lobes were ruptured by gently pressing on the coverslip with the point of a dissecting needle. The surrounding fluid was then searched for sporozoites. When free in the saline solution their peculiar behaviour made identity clear. In all doubtful cases a stained preparation was made before making a diagnosis. Excluding the initial period of about six weeks, when, through lack of trained assistance, the majority of examinations were of the alimentary tract only, every endeavour was made to examine the stomach and salivary glands of each specimen, though a successful dissection of both organs was not always achieved.

III. SURVEY OF KAMPALA

Kampala, the commercial capital of Uganda, is situated at an altitude of 3,900 feet, five miles north-west of Lake Victoria, on a number of hills with intervening swampy valleys and sluggish streams, and in the centre of a thickly populated native area. Malaria is hyperendemic amongst the indigenous population. Dr. N. C. Macleod (1930) was able in three examinations to demonstrate parasites of malaria in all of eighteen native children under ten years of age, living in the selected observation huts where regular collections of mosquitoes were made. On the first occasion fourteen were positive, three more showed parasites on the second examination, and the remaining one was positive on the third examination.

Six species of *Anopheles*—*costalis*, *funestus*, *theileri* var. *hancocki*,* *mauritanus*, *squamosus* and *implexus*—were found breeding within and around the township. Of these the three former were proved by dissection to be concerned in the dissemination of malaria. *Anopheles costalis* and *funestus* were very common and represented 50·7 per cent. and 43·4 per cent. respectively of the total female *Anopheles* caught in houses. *A. theileri* (5·9 per cent.) was not so widely

* All the specimens of *Anopheles theileri* dissected and referred to in this paper belong to var. *hancocki*. Typical *A. theileri* have recently been found in Uganda, but no dissections of this form have been made. The two forms differ widely in both adult and larval characters, and it is therefore important to emphasize to which variety the material examined belongs.

distributed and showed a preference for one locality. Neither *implexus* nor *squamosus* frequented human dwellings, while a single adult only of *mauritanus* was captured in a native hut.

(a) DISTRIBUTION OF *ANOPHELES*

For the purpose of this survey, catches of adult mosquitoes were made regularly, twice weekly, in seventy-two selected native huts situated in six different localities in the neighbourhood of the town. It was so arranged that four huts in each locality were searched daily. Their construction rendered it impracticable to clear them absolutely of mosquitoes on each visit, but every attempt was made to catch as many as possible. The huts were of the mud and wattle type, and extremely dark inside. Most of them were divided into a number of small windowless rooms, which, in turn, if used for sleeping in and occupied by more than one person, were subdivided by hanging cloth from wall to wall. Even in the heat of the mid-day sun the inner rooms were pleasantly cool and provided a sheltered retreat for the mosquito, particularly when it was customary for the occupants to light their fires and do the cooking in the outer rooms. Though it was the practice to search the huts in the early morning and forenoon, it was noted that they were just as productive if searched in the afternoon.

During the year's survey 22,463 mosquitoes, of which 79 per cent. were *Anopheles*, were caught in the observation huts. Fig. 1 shows the total number of female *Anopheles* caught in each locality correlated with the percentage infected. A striking feature is the comparatively high infection rate in localities in which a small number were caught. This may be accounted for by the fact that the observation huts in some localities were nearer breeding places, and a greater percentage of newly-emerged adults, which had not had time to become infected, were among those collected. Also the presence of *Anopheles* in large numbers makes the inhabitants restive; the occasional furtive mosquito in the less heavily infected areas would have much more opportunity of obtaining a good feed and thereby of imbibing gametocytes. Observations conducted in my own house situated in the European residential area, where *Anopheles* were comparatively rare, also showed a high infection rate. Of four female *Anopheles*, all *costalis*, caught in the course of eight months, two were found infected

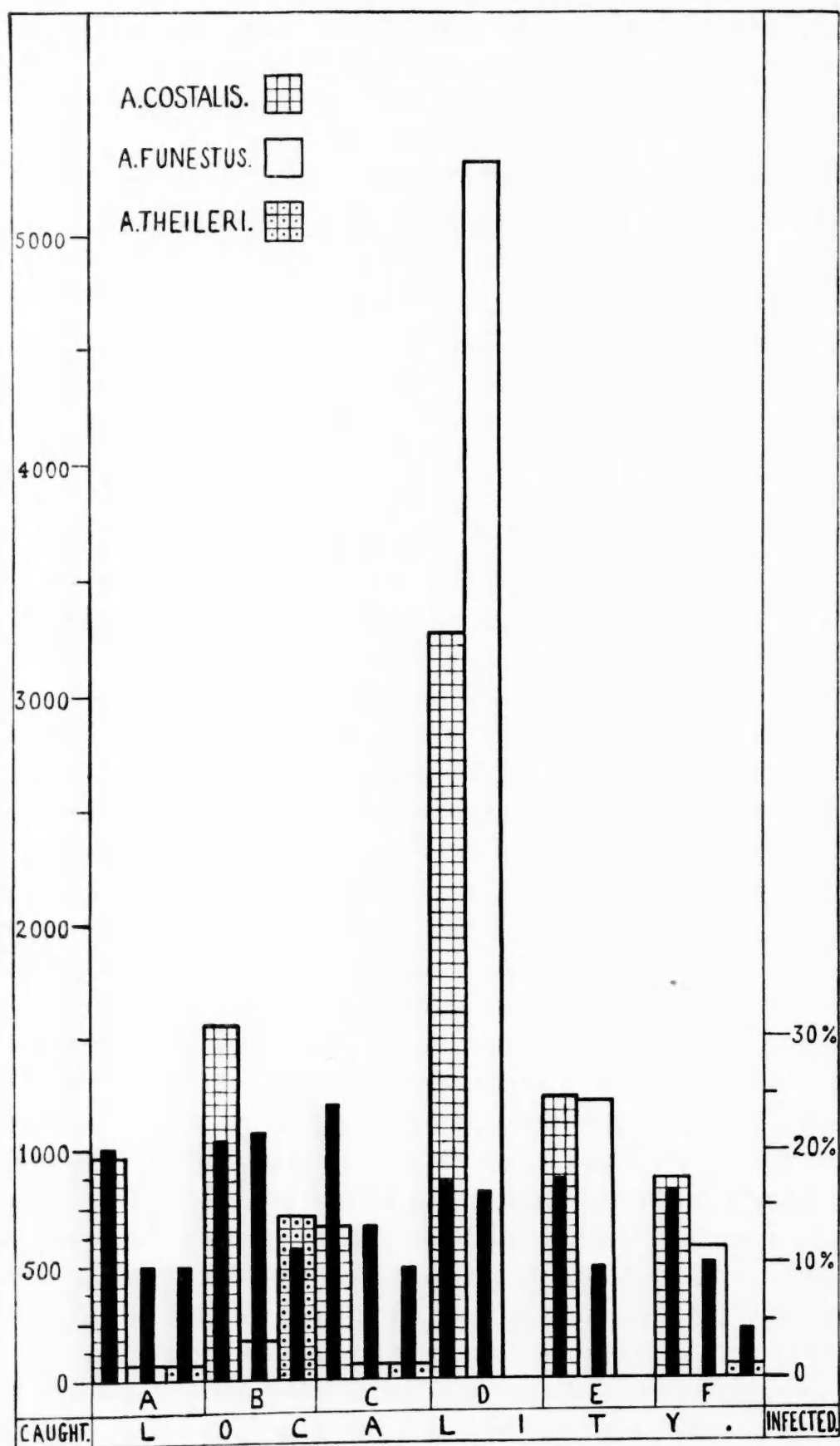


FIG. 1. Showing the total number of female *Anopheles* caught in each locality in Kampala, correlated with the percentage infected, which is superimposed in black.

on dissection; one of these had a single oöcyst on the stomach wall, and the other was heavily infected with sporozoites in the salivary glands. It will be seen that *theileri* was not common in localities other than B, but wherever it occurred some were found infected. Man was responsible for the overwhelming abundance of *funestus* and *costalis* in locality D, where the excavation of clay for brick-making had provided pits especially suitable for these two species.

(b) INFECTION OF *ANOPHELES*

The result of dissections of the alimentary tract and salivary glands is shown in detail in Table I and summarised below:

Species of <i>Anopheles</i>	Number examined		Stomach infected (oöcysts)		Salivary glands infected (sporozoites)		Double infection (stomach and glands infected)		Percentage infected
	Stomach	Salivary glands	No.	%	No.	%	No.	%	
<i>A. costalis</i>	6,836	6,486	701	10.2	653	10	108	1.6	18.6
<i>A. funestus</i>	5,853	5,665	462	7.8	469	8.2	69	1.2	14.8
<i>A. theileri</i> var. <i>bancrofti</i>	1,014	983	92	9	27	2.7	3	0.3	11.4

It will be seen that the monthly infection rate fluctuated between 14.3 per cent. and 24.9 per cent. in *costalis*, 13 per cent. and 17.7 per cent. in *funestus* and 0 and 19.9 per cent. in *theileri*. *Anopheles costalis* showed the maximum sporozoite infection of the salivary glands in June (15.7 per cent.), *funestus* in December (9.4 per cent.) and *theileri* in February (7 per cent.). Double infections (i.e., oöcysts on the stomach wall and sporozoites in the salivary glands of one and the same mosquito) were seldom encountered in *theileri*, but were quite common in *costalis* and *funestus*. In the majority of cases the sporozoites were present in large numbers. There was a tendency for them to collect in different parts of the glands, and the different lobes were not always affected to the same degree; on several occasions one lobe was seen to be heavily infected while others appeared normal. Stained preparations of the sporozoites showed the morphological differences illustrated in fig. 2, *a*. Table II is a

statement of the number of oöcysts present on the stomach of the individual mosquito. Single oöcysts were more common in *costalis* and *theileri* than in *funestus*. Three specimens (0.4 per cent.) of *Anopheles costalis* were found with over one hundred oöcysts (one



FIG. 2. Intestinal parasites of *Anopheles* other than malaria.

showed 152), only one *funestus* (0.2 per cent.), while in the case of *theileri* only one stomach showed more than twenty. It was noted that oöcysts were present in the presence of bacteria, flagellates and other cysts.

(c) STUDY OF THE OÖCYST

Fig. 3 illustrates the types of oöcysts met with in the course of this investigation. The figures are all drawn to the same scale and depict the oöcyst as seen in the fresh state. The characteristic pigment of all three forms of human malaria was observed during this study. Oöcysts with fine wisps of golden pigment, as depicted from the stomach of *costalis* and *funestus* (fig. 3, *c* and *d*), were easily recognised as *Plasmodium vivax* (Wenyon, 1926), and those containing the dark brown pigment of *Plasmodium falciparum*, in the form of pepper-like grains, presented no difficulty, but their differentiation from the coarse pigmented oöcysts of *Plasmodium malariae* when the grains had become agglutinated into an irregular mass, as apparently occurs at a certain stage in the cyst's development, was often an impossibility. In the single oöcyst (fig. 3, *b*, right-hand cyst), the two types of pigment are demonstrated: the one vacuole contains an irregular mass, evidently the result of agglutination of separate particles, whereas in the neighbourhood of the other the pigment is in the form of individual granules. There is reason to suppose that a similar phenomenon takes place in the case of *Plasmodium malariae*, the coarse masses seen in one of the oöcysts depicted in fig. 3, *g*, which on careful focusing proved to be composed of single rods of pigment, appear to support this hypothesis. Mixed infections were not uncommon in all three species of *Anopheles*. Fig. 3, *g*, shows a mixed infection of *Plasmodium falciparum* and *P. malariae* in *costalis*, and fig. 3, *i*, *Plasmodium vivax* and *P. malariae* in *funestus*. The two oöcysts from *theileri* (fig. 3, *f*) may represent a mixed infection of *Plasmodium falciparum* and *P. malariae* or a single infection of the former species, the individual grains in the one cyst having collected together to form four unequal masses of pigment. Two of the 152 oöcysts of different ages from the stomach of *costalis* are illustrated in fig. 3, *h*. Pigment was either absent or invisible in a large number of cysts, while in the others it was represented by a few grains resembling *Plasmodium falciparum*. The coarse rods of dark pigment exhibited in the three small oöcysts (fig. 3, *e*) were seldom encountered

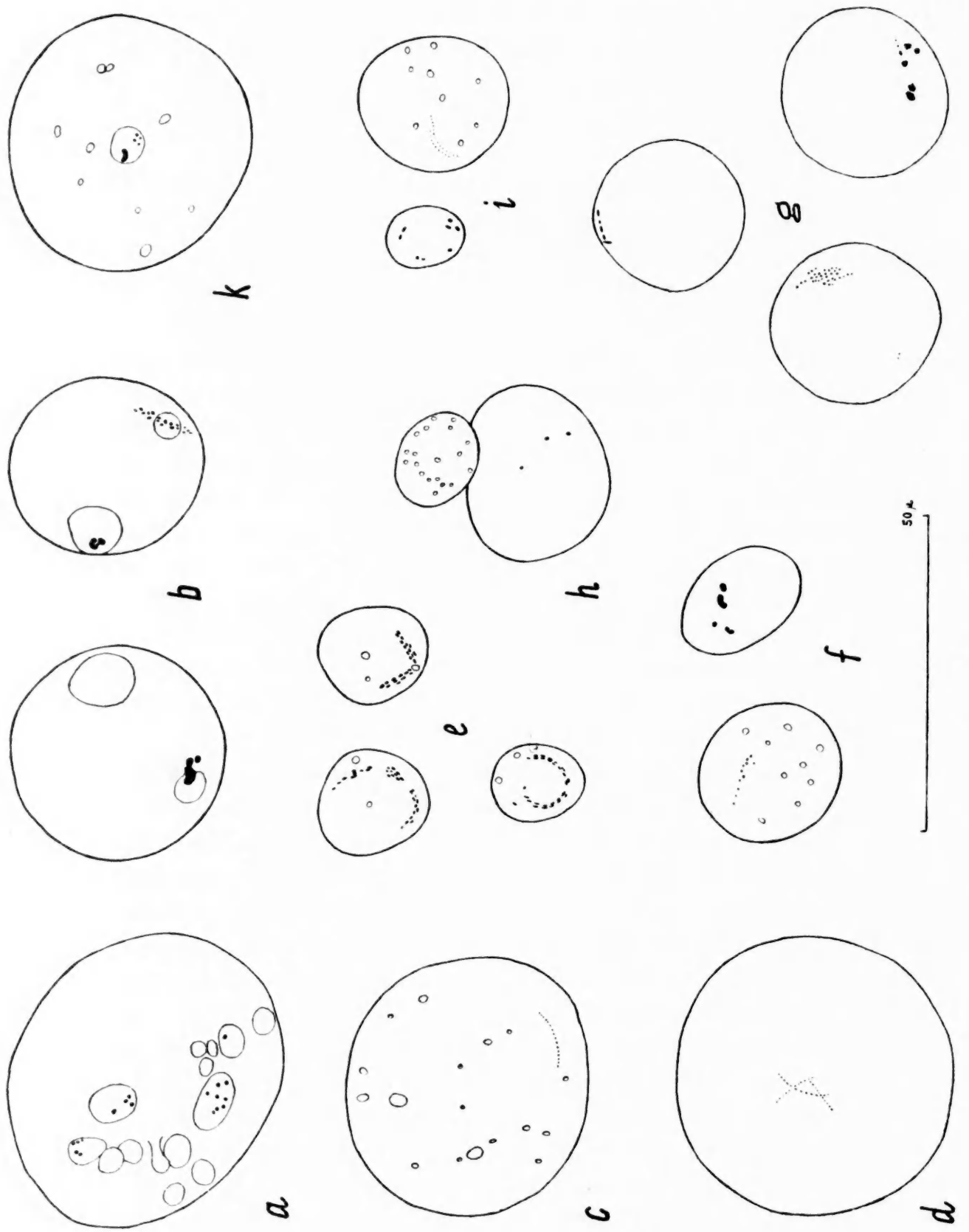


Fig. 3. Types of Oöcysts.

in large cysts, and it seems highly probable that at a later stage in their development the individual rods adhere together, forming coarse masses of pigment not unlike *Plasmodium malariae*. A peculiar phenomenon was observed in one of seventeen oöcysts on the alimentary tract of *costalis* (fig. 3, k): within a central vacuole were a very active oscillating dark brown bean-shaped body and four dormant grains of pigment.

(d) CLIMATIC FACTORS INFLUENCING THE SEASONAL PREVALENCE AND INCIDENCE OF INFECTION OF *ANOPHELES COSTALIS* AND *ANOPHELES FUNESTUS*.

Monthly prevalence, incidence of infection and rainfall are correlated in fig. 4. *Anopheles costalis* exhibits a remarkable peak of

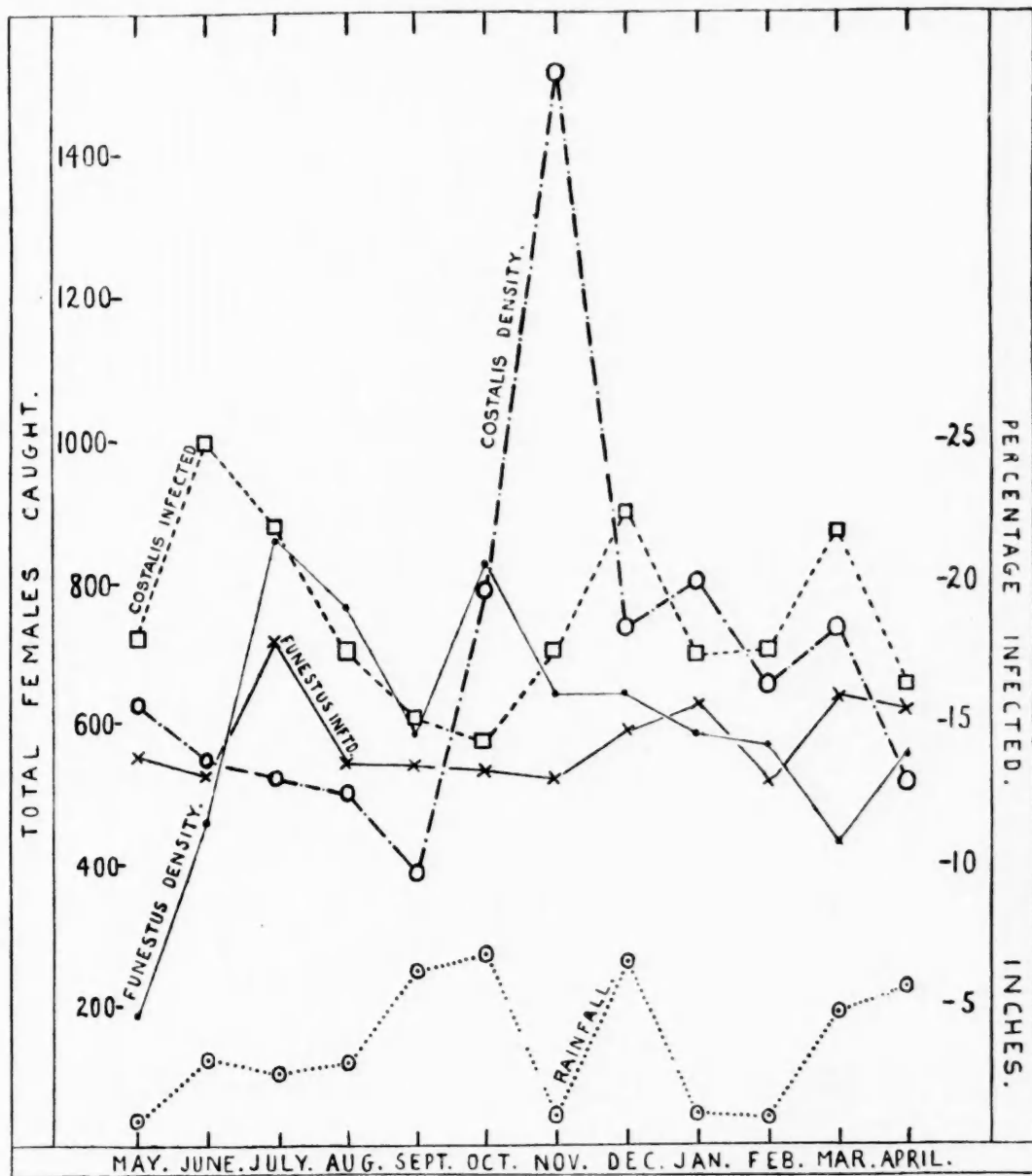


FIG. 4. Correlation of monthly prevalence of *A. costalis* and *A. funestus* with incidence of infection and rainfall.

increased density following the heavy rains of September and October. That this species is mainly dependent on rainfall for the creation of suitable breeding places in Kampala is clearly seen. When, in the early months of this investigation, there was little rain, a gradually diminishing number was recorded. Quite a different picture is presented by the curve of *funestus*. Two similar peaks of maximum density occur within four months, the first in the dry season and the second shortly after the onset of the long rains. The curves for infection have been placed with those for density, but it is questionable whether a direct relationship exists.

Mean relative humidity, dry bulb temperature and incidence of infection are shown in fig. 5. It is very evident that a direct relationship exists between the incidence of malaria infection in *costalis* and

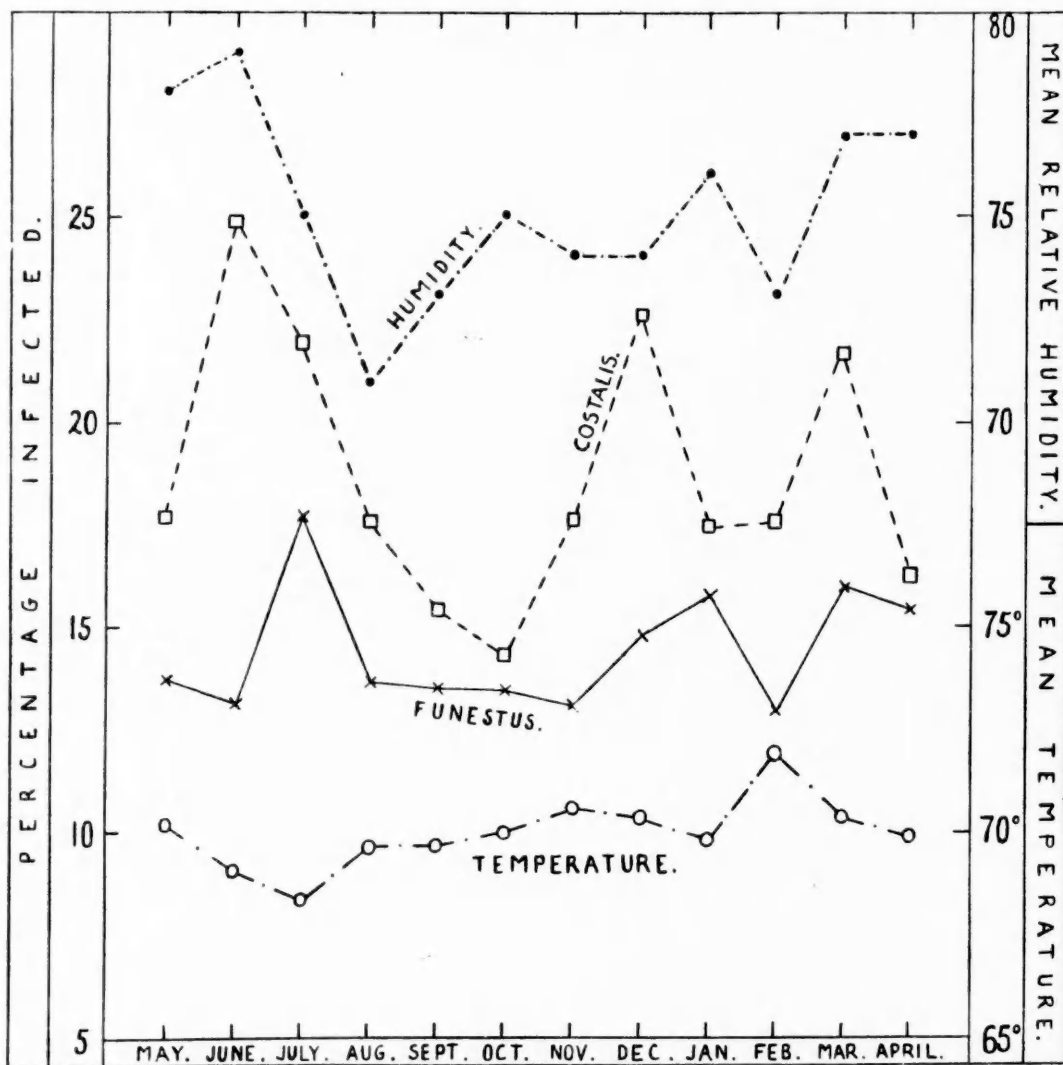


FIG. 5. Correlation of mean relative humidity, dry bulb temperature and incidence of infection of *A. costalis* and *A. funestus*.

funestus, and humidity and temperature. The influence of humidity is apparent in both species, whereas the effect of temperature is more pronounced in the case of *funestus*. By inverting the temperature curve, one reproduces an almost exact representation of the infectivity curve of this species. Both *costalis* and *funestus* show a rise in incidence following a drop in temperature.

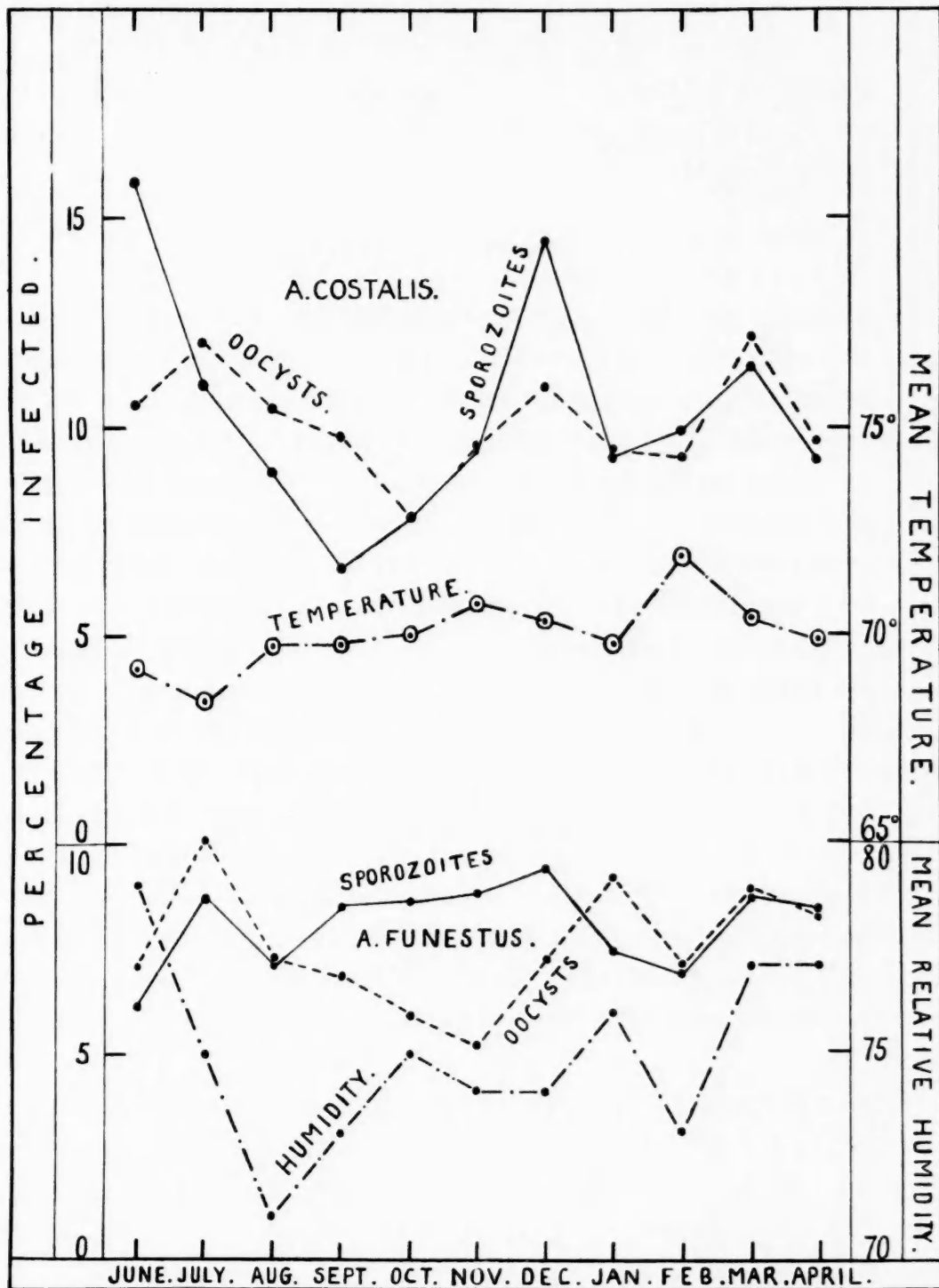


FIG. 6. Correlation of dry bulb temperature, mean relative humidity and sporozoite and oöcyst rates of *A. costalis* and *A. funestus*.

In fig. 6, the curves for dry bulb temperature and mean relative humidity are correlated with those for the monthly oöcyst and sporozoite rates. The influence of temperature on sporozoite production is clearly seen in both species. Its effect is most marked in the case of *funestus*, where the curve for sporozoites is seen to forsake the course of that for oöcysts, and to follow the gradually ascending curve of temperature for a period of four months. The climatic conditions of December were most favourable to the development of sporozoites in both species. This month followed a dry interval in a rainy spell, when temperature was on the decline and humidity stable.

IV. SURVEY OF JINJA

The survey of Jinja, a town situated on high ground about 3,800 feet above sea level, overlooking Lake Victoria at the source of the River Nile, was commenced in June, 1930, and carried on over a period of four months, which almost coincided with the dry season.

The mosquito fauna found breeding in this region included *Anopheles funestus*, *costalis*, *pharoensis*, *moucheti*, *mauritanus*,* *nili*, *symesi*, *squamosus* and *implexus*, of which the first four were demonstrated by dissection to be carriers of parasites of malaria. *Anopheles funestus* was the predominant species, *costalis* occurred in smaller numbers, *pharoensis* was local and not very common, and *moucheti* confined its activities to one area. *Anopheles mauritanus* habitually entered human dwellings in Jinja, and was commonly taken engorged with blood, though it was not found infected with plasmodia of malaria. Its occurrence in native huts in unusual numbers coincided with the presence of goats. *Anopheles nili* was taken on rare occasions in association with *moucheti*, but was not concerned in the transmission of the disease in Jinja. Of the remaining three species, a single adult *implexus* was caught, which proved negative on dissection.

The importance of carrying out an adult as well as a larval survey to ascertain the prevalent species in a district was strikingly revealed at Jinja, where the predominant larva was *costalis* and the predominant adult *funestus*. Very seldom, and only after prolonged

* All the specimens of *A. mauritanus* dissected at Jinja belong to var. *ziemanni*.

dipping in the reeds along the lake shore, was a larva of *funestus* obtained, while in human dwellings this species was exceedingly common and represented 59 per cent. of the *Anopheles* present, as opposed to 10.7 per cent. of *costalis*.

(a) DISTRIBUTION OF *ANOPHELES*

Throughout the survey 108 selected native huts situated in six different localities in and around the township, as well as screened European houses and their servants' quarters, were searched twice weekly for mosquitoes. Of a total of 18,352 mosquitoes collected in human dwellings, 82 per cent. were *Anopheles*. The female specimens were distributed numerically as follows:—*Anopheles funestus* 8,986 (59 per cent.), *moucheti* 4,183 (27.4 per cent.), *costalis* 1,643 (10.7 per cent.), *mauritanus* 244 (1.5 per cent.), *pharoensis* 157 (1 per cent.), *nili* 14, and *implexus* 1.

The collections showed clearly that, whereas huts in close proximity to the lake and river are heavily infested with mosquitoes, others situated some distance inland are practically free. A striking feature was the overwhelming preponderance of *funestus* in huts situated near the lake, and its almost complete absence in the vicinity of the River Nile, where it was replaced by *moucheti*.

The following table shows the distribution of the species in European premises.

Situation	SPECIES OF <i>Anopheles</i>				
	<i>A. costalis</i>	<i>A. funestus</i>	<i>A. moucheti</i>	<i>A. pharoensis</i>	<i>A. mauritanus</i>
European houses	12	182	11	2	10
Servants' quarters	31	676	8	1	3

The dominant species in European houses was *funestus*, which was found infected to the extent of 3.6 per cent. Excepting *nili*, all the species found habitually to enter human abodes in the neighbourhood of Jinja were at one time or other taken in European houses. Of two specimens of *pharoensis*, one showed a single oöcyst on the stomach wall. The mosquitoes taken in servants' quarters situated

in the compounds of European houses were recorded separately, and showed that 87 per cent. of the malaria-carrying anophelines caught in the European residential area came from this situation. These quarters, while in themselves light and airy, were rendered very dark and particularly attractive to anophelines by curtaining off the rooms into small sections and obstructing the only window. Of sixteen specimens of *costalis* dissected, six were infected, while the infection rate of *funestus* was 6.2 per cent.

(b) INFECTION OF *ANOPHELES*

During the four months, 5,897 alimentary tracts and 5,922 salivary glands were examined, particulars of which are given in Table III and summarised below.

Species of <i>Anopheles</i>	Number examined		Stomach infected (oöcysts)		Salivary glands infected (sporozoites)		Double infection (stomach and glands infected)		Percentage infected
	Stomach	Salivary glands	No.	%	No.	%	No.	%	
<i>A. costalis</i>	798	799	97	9.8	83	10.3	19	2.3	17.8
<i>A. funestus</i>	3,645	3,666	199	5.1	172	4.6	21	0.5	9.2
<i>A. moucheti</i>	1,059	1,061	61	5.7	31	2.9	4	0.3	8.3
<i>A. pharoensis</i>	150	151	8	5.3	1	0.6	5.9
<i>A. mauritanus</i>	237	237
<i>A. nili</i>	7	7
<i>A. implexus</i>	1	1

The monthly incidence of infection fluctuated between 14.9 per cent. and 27.5 per cent. in the case of *costalis*, 7.1 per cent. and 11.9 per cent. in *funestus*, 6.1 per cent. and 11.7 per cent. in *moucheti*, and 2.7 per cent. and 8 per cent. in *pharoensis*. *Anopheles costalis* showed the maximum sporozoite infection of the salivary glands in August (16.1 per cent., of which 5.6 per cent. showed oöcysts on the stomach as well), *funestus* in December (6.8 per cent.), *moucheti* in June (4.1 per cent.), while in the case of *pharoensis* the presence of

sporozoites was only detected on one occasion, in August, out of 151 specimens dissected. *Anopheles mauritianus* and *nili* proved negative to parasites of malaria. From Table II it will be seen that single oöcysts were most common in *funestus*, the predominant species. In this species seventy-seven oöcysts were seen on one occasion. Neither *costalis* nor *moucheti* showed more than fifty, and six was the largest number counted in *pharoensis*.

V. SURVEY OF FORT PORTAL

The station of Fort Portal, through which the River Mpanga meanders, is situated near the Ruwenzori mountains, at an altitude of 5,171 feet above sea level, and is surrounded by extensive swamps. Its cool climate and cloudy atmosphere is not congenial to the sun-loving *Anopheles* of lower altitudes. The survey, commenced in September, 1931, was continued for five months and included the wet season; September and January were dry, but during the other months rain was almost a daily occurrence. A single blood examination of twenty-nine native children under ten years of age residing in the observation huts showed that three were harbouring parasites of malaria.

Among the mosquitoes found breeding were the following *Anopheles*:—*marshalli*, *funestus*, *costalis*, *transvaalensis*, *mauritianus* and *christyi*. *Anopheles marshalli*, a well-marked local form, was the prevalent species in houses, and together with *funestus* was incriminated in the rôle of transmitting malaria in Fort Portal. *Anopheles transvaalensis*, a common mosquito in houses in the district, did not harbour malarial parasites, and *costalis*, the important carrier in other parts of the country, played no part in the transmission of the disease during the survey. *Anopheles mauritianus* and *christyi* were not taken in human habitations.

(a) DISTRIBUTION OF *ANOPHELES*

During the survey, sixty native huts, situated in five different localities within and near the township, were searched regularly for mosquitoes. Of the specimens collected, 93 per cent. were *Anopheles*.

The species were found to be distributed numerically as follows :— 997 (49·3 per cent.) *marshalli*, 557 (27·5 per cent.) *funestus*, 453 (22·4 per cent.) *transvaalensis*, and 14 (0·7 per cent.) *costalis*. Both *marshalli* and *transvaalensis* were common in European houses in December and January, and, when found in unusual numbers in native huts, indicated the presence of domestic animals. *Anopheles funestus* did not occur in European premises and confined itself to huts in one locality.

(b) INFECTION OF *ANOPHELES*

The accompanying table gives a summary of the dissections carried out during the survey.

Species of <i>Anopheles</i>	Number examined		Stomach infected (oöcysts)		Salivary glands infected (sporozoites)		Double infection (stomach and glands infected)		Percentage infected
	Stomach	Salivary glands	No.	%	No.	%	No.	%	
<i>A. marshalli</i> ...	914	923	1	0·1	2	0·2	0·3
<i>A. funestus</i> ...	552	553	20	3·6	8	1·4	5
<i>A. transvaalensis</i> ...	430	435
<i>A. costalis</i> ...	11	11

The infectivity rate of *funestus* is low in comparison with that found in other places in Uganda. It will be seen that only a small percentage exhibited sporozoites in the salivary glands. The number of oöcysts present on the stomach of the individual mosquito in the twenty infected was : one in seven, from two to ten in six, up to twenty in five, and a single specimen with twenty-six. Three examples of *marshalli* showed plasmodia of malaria ; in two cases the glands were heavily charged with sporozoites, while in the third instance a single oöcyst was present on the stomach. The few dissections of *costalis* and all those of *transvaalensis* proved negative on examination.

VI. SURVEY OF MBALE

A short infectivity survey of the *Anopheles* of Mbale was carried out during the month of April, 1932, which was almost equally divided into a wet and dry period. The town is situated at the edge of the Bugwere plains, below Mt. Elgon, and between two mountain streams, at an altitude of 4,000 feet.

Three species of *Anopheles*—*costalis*, *funestus* and *mauritanus*—were found breeding within and around the township. Of these the two former, which were extremely common in native huts in one locality and present in about equal numbers, were found to be heavily infected with parasites of malaria. *Anopheles mauritanus* was not taken in human dwellings.

Details of the dissections carried out are tabulated below :—

Species of <i>Anopheles</i>	Number examined		Stomach infected (oöcysts)		Salivary glands infected (sporozoites)		Double infection (stomach and glands infected)		Percentage infected
	Stomach	Salivary glands	No.	%	No.	%	No.	%	
<i>A. costalis</i>	109	114	16	14·6	12	10·5	1	0·9	24·2
<i>A. funestus</i>	112	112	5	4·4	6	5·3	9·7

Anopheles costalis shows a high infection rate. In two instances the stomach of this species exhibited 102 and 205 small oöcysts on the stomach wall. On one occasion forty-three oöcysts were present on the alimentary tract, while the salivary glands were heavily charged with sporozoites. *Anopheles funestus* was not so heavily infected, but nevertheless showed 5·3 per cent. with gland infections.

VII. INTERNAL PARASITES OTHER THAN MALARIA

Parasites other than malaria met with in the course of this inquiry are shown in the following table.

Parasite	Organ of mosquito	Species of <i>Anopheles</i>	Number of cases
Microfilariae	Stomach	<i>costalis</i> ...	2
		<i>theileri</i> ...	2
Other nematodes	Thorax	<i>costalis</i> ...	7
		<i>funestus</i> ...	14
		<i>theileri</i> ...	2
	Thorax and proboscis	<i>funestus</i> ... <i>mauritanus</i> ...	1 1
Nematode ova	Ovaries	<i>costalis</i> ...	1
Fish-like bodies	Stomach	<i>costalis</i> ...	8
		<i>funestus</i> ...	2
	Thorax	<i>costalis</i> ... <i>funestus</i> ...	208 31
Flagellates	Stomach	<i>costalis</i> ...	15
		<i>funestus</i> ...	16
		<i>theileri</i> ...	9
		<i>mauritanus</i> ...	2
		<i>moucheti</i> ...	3
		<i>transvaalensis</i> ...	7
Cysts	Stomach	<i>costalis</i> ...	12
		<i>funestus</i> ...	55
Ciliates	Ovaries	<i>funestus</i> ...	6
		<i>theileri</i> ...	2
Ovoid bodies	Ovaries	<i>costalis</i> ...	6
		<i>funestus</i> ...	25

Microfilariae. Sheathed microfilariae were seen in the alimentary tract of four specimens—two *costalis* and two *theileri*.

Other nematodes. Active embryos resembling microfilariae were seen in *costalis*, *funestus*, *theileri* and *mauritanus*. They occurred singly, excepting on five occasions when two, three, four, five and ten respectively were present in the thorax. In two instances, in addition to embryos in the thorax, others were seen in the proboscis. The labium of *mauritanus* showed two nematodes side by side, while three more were found in the thoracic muscles. In the case of *funestus*, a single specimen was seen in the act of leaving the proboscis.

Nematode ova. Many objects resembling ova of *Ascaris* were seen in the ovaries of one *costalis*.

Fish-like bodies. The peculiar bodies illustrated in fig. 2, *b*, were found on numerous occasions in the thorax, and were often observed leaving the salivary glands after rupture. They resemble a sporozoon and occurred in small numbers, seldom more than twenty. In the fresh state they are pale green, glistening, fish-shaped bodies, feebly motile, containing refractile granules and varying in length and breadth from 15μ to 20μ and from 2μ to 5μ .

Flagellates. These are *Leptomonas* forms and were found in large numbers in the stomach. The various forms seen are illustrated from *gambiae*, in fig. 2, *c*, and from *theileri*, in fig. 2, *d*, *e* and *f*.

Cysts. The cysts contained spores and varied much in shape and size. They were commonly found in large numbers, often covering the whole stomach.

Ciliates. Active ciliates resembling *Chilodon cullulus* were seen on six occasions.

Ovoid bodies. These were very numerous in the ovaries. They were light brown in colour, varied from 25μ to 30μ in length, and consisted of a finely granular homogeneous oval body within an outer shell.

VIII. EXAMINATION OF DOMESTIC CULICINES

In the course of the surveys of Kampala and Jinja, culicines, taken along with the anopheline mosquitoes, were invariably found engorged with blood. Time did not permit more than a small number of dissections of the predominant species, but the results of those examined may not be without interest. In Kampala the alimentary tract, thorax and proboscis of twenty-two *Culex fatigans* Wied. were examined, and one exhibited a sheathed microfilaria in the gut. During August and September, 1930, 102 specimens of *Mansonia uniformis* Theo., the most troublesome culicine in Jinja, were dissected. Fourteen active nematodes resembling embryos of microfilaria were found in the thorax of one insect. The stomach and salivary glands showed no plasmodia of bird malaria. Other culicines caught in human habitations, of which no dissections were made, included *Aedes aegypti*, *A. lineatopennis* Ludl., *A. tarsalis* Newst., *Culex nebulosus* Theo., *C. cinereus* Theo., *C. musarum* Edw., *C. laurenti* Newst., *C. pipiens* L., *C. horridus* Edw., *Mansonia fuscipennatus* Theo. and *M. africanus* Theo.

IX. SUMMARY

Of the six species of *Anopheles* incriminated as vectors of malaria in Uganda, two only, *costalis* and *funestus*, were encountered in more than one region.

1. *Anopheles costalis* is the most dangerous malaria-carrying species in the country. At Kampala, 6,836 stomachs and 6,486 salivary glands of this species were examined, and 18.6 per cent. showed infection with plasmodia of malaria. During the year's survey the monthly infection and sporozoite rates fluctuated between 14.3 per cent. and 24.9 per cent., and 5.8 per cent. and 15.7 per cent. respectively. At Jinja, 798 stomachs and 799 salivary glands were examined, and 17.8 per cent. were infected. In this township, during a period of four months, the infection and sporozoite rates fluctuated between 14.9 per cent. and 27.5 per cent., and 7.1 per cent. and 16.1 per cent. respectively. At Mbale, in April, out of 109 stomachs and 114 glands examined, 24.2 per cent. were infected, of which 10.5 per cent. were gland infections. The distribution of this species in Uganda appears to be governed principally by temperature. The species was common about the warm sunny towns of 4,000 feet elevation, whereas at Fort Portal (5,000 ft.), during the wet season when the prevailing temperature was comparatively low, in spite of the presence of numerous apparently suitable breeding places, it was seldom found. When the temperature is favourable, its prevalence is greatly influenced by rainfall.
2. *Anopheles funestus* is the most abundant malaria-carrying species in Uganda. In all the regions surveyed, it was found to harbour parasites of malaria. At Kampala, 5,853 stomachs and 5,665 salivary glands were examined, and 14.8 per cent. were found infected. The infection and sporozoite rates fluctuated between 13 per cent. and 17.7 per cent., and 6.1 and 9.4 per cent. respectively. At Jinja, 1,059 stomachs and 1,061 glands showed 9.2 per cent. infected. Here the infection and sporozoite rates fluctuated between 7.1 per cent. and 11.9 per cent., and 3 per cent. and 6.8 per cent.

respectively. At Fort Portal, out of 552^{stomachs} and 553 glands examined, 5 per cent. were infected, of which 1.4 per cent. were gland infections. At Mbale, 112 stomachs and 112 glands showed 9.7 per cent. infected, of which 5.3 per cent. were gland infections.

3. *Anopheles theileri* var. *hancocki* was found to be an important vector of malaria in one locality in the neighbourhood of Kampala. Out of 1,014 stomachs and 983 salivary glands examined, 11.4 per cent. were found infected. The monthly infection and sporozoite rates fluctuated between 0 and 19.9 per cent., and 0 and 7 per cent. respectively.
4. *Anopheles moucheti* was common in the vicinity of the River Nile at Jinja, where it was found to be of considerable importance as a carrier of parasites of malaria. An examination of 1,059 stomachs and 1,061 salivary glands showed 8.3 per cent. with malaria infection. The infection and sporozoite rates of this species fluctuated between 6.1 per cent. and 11.7 per cent., and 1.6 per cent. and 4.1 per cent. respectively.
5. *Anopheles marshalli* occurred in the high country surrounding Fort Portal, and of 914 stomachs and 923 salivary glands examined during five months 0.3 per cent. were found infected, 0.2 per cent. with gland infections.
6. *Anopheles pharoensis* was limited in its distribution, and occurred in numbers only in the near vicinity of its breeding places on the lake shore at Jinja. Of 150 stomachs and 151 salivary glands examined during four months, 5.9 per cent. were infected, of which 0.6 per cent. were gland infections.
7. *Anopheles mauritanus* and *transvaalensis*, which habitually frequent human dwellings in Jinja and Fort Portal respectively, were commonly taken engorged with blood, but were not found to transmit malaria. The number of specimens dissected (237 in the case of the former, and 435 in the latter) is sufficient to justify the conclusion that they are not concerned in the dissemination of malaria in the towns surveyed. The presence of these two species in unusual numbers in native huts almost always coincided with the presence of goats, and it is probable that they prefer animal to human blood. That these species do imbibe human

blood is undoubted: the writer caught specimens of these mosquitoes in the act of biting him, three times in the case of *mauritanus* and once in the case of *transvaalensis*.

8. *Anopheles nili* was taken on rare occasions in native huts in association with *moucheti* in the vicinity of the River Nile. Of fourteen specimens collected, seven were dissected, but none showed infection with plasmodia of malaria. Schwetz (1929) states that he found this species to be a vector of malaria in Stanleyville.
9. The study of pigmented oöcysts occurring in *Anopheles costalis*, *funestus* and *theileri* at Kampala suggests that each of these species is concerned in the spread of all three forms of human malaria. The recognition of oöcysts of more than one species on the stomach of a single *Anopheles* indicates the possibility of the individual insect transmitting mixed infections.
10. That maturation of oöcysts takes longer in *Anopheles theileri* var. *hancocki*, *moucheti* and *pharoensis* than in *costalis* and *funestus* is indicated by the considerably smaller percentage of gland to stomach infections recorded in the former species.
11. The heaviest intestinal infections were recorded in *Anopheles costalis*. This species exhibited over one hundred oöcysts on four occasions. In one insect 205 small oöcysts were counted.
12. It is probable that *Anopheles funestus* may play the more important part in the causation of malaria at certain seasons in Kampala, owing to acceleration in the development of sporozoites at a period when this phenomenon is retarded in *costalis*.

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TABLE I

Showing monthly dissections and the infection rate of the *Anopheles* of Kampala

Species of <i>Anopheles</i>	Month	Number examined		Stomach infected (oocysts)		Salivary glands infected (sporozoites)		Double infection (stomach and glands infected)		Percentage infected
		Stomach	Salivary glands	No.	%	No.	%	No.	%	
<i>A. costalis</i>	May	329	119	40	12.1	7	5.8	17.9
	June	425	222	45	10.5	35	15.7	3	1.3	24.9
	July	441	443	53	12	49	11	5	1.1	21.9
	August	416	413	44	10.5	37	8.9	8	1.9	17.5
	September	387	393	38	9.8	26	6.6	5	1.2	15.2
	October	723	737	56	7.7	57	7.7	8	1.1	14.3
	November	1,136	1,184	108	9.5	112	9.4	16	1.4	17.5
	December	641	651	71	11	94	14.4	19	2.9	22.5
	January	636	644	61	9.5	60	9.3	10	1.5	17.3
	February	586	594	55	9.3	59	9.9	10	1.7	17.5
	March	586	588	72	12.2	68	11.5	12	2	21.7
	April	442	442	43	9.7	41	9.2	12	2.7	16.2
<i>A. funestus</i>	May	102	39	14	13.7	13.7
	June	314	178	22	7	11	6.1	13.1
	July	661	631	67	10.1	55	8.7	7	1.1	17.7
	August	610	598	45	7.3	43	7.1	5	0.8	13.6
	September	582	595	40	6.8	51	8.5	11	1.8	13.5
	October	762	785	45	5.9	68	8.6	9	1.1	13.4
	November	398	409	21	5.2	36	8.8	4	1	13
	December	506	509	37	7.3	48	9.4	10	1.9	14.8
	January	413	415	38	9.2	31	7.4	4	0.9	15.7
	February	491	498	35	7.1	34	6.8	5	1	12.9
	March	521	524	46	8.8	46	8.7	9	1.7	15.8
	April	486	486	40	8.2	41	8.4	6	1.2	15.4
<i>A. theileri</i>	May	30	30
	June	91	67	1	1.4	1.4
	July	131	127	9	6.8	2	1.5	8.3
	August	91	76	10	10.9	10.9
	September	103	102	8	7.7	2	1.9	9.6
	October	76	80	7	9.2	1	1.2	10.4
	November	65	65	6	9	1	1.5	10.5
	December	59	59	2	3.3	1	1.6	4.9
	January	52	53	4	7.6	1	1.8	9.4
	February	100	100	7	7	7	7	1	1	13
	March	95	95	15	15.7	5	5.2	1	1	19.9
	April	116	116	12	10.3	6	5.1	1	0.8	14.6

TABLE II

Oöcysts	<i>Anopheles costalis</i>				<i>Anopheles funestus</i>				<i>Anopheles theileri</i>		<i>Anopheles mouchei</i>	
	Kampala		Jinja		Kampala		Jinja					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	319	48.3	39	51.3	103	29	116	57.1	44	52.3	30	49.1
2	100	15.1	12	15.7	85	24	32	15.6	21	25	10	16.4
3-5	129	19.6	12	15.7	80	22.6	39	19.1	8	9.5	9	14.7
6-9	43	6.5	5	6	44	12.1	8	3.4	6	7.1	5	8.2
10-49	61	9.2	8	10.5	34	9	8	3.4	5	5.9	7	11.4
50-100	5	0.7	7	2	1	0.4
Over 100	3	0.4	1	0.2

TABLE III

Showing the monthly dissections and the infection rate of the *Anopheles* of Jinja

[illegible]

Data from the Kampala Meteorological Station

Month	Mean dry bulb temperature	Mean relative humidity	Total rainfall (inches)
May	70.2	78	0.8
June	69.1	79	3.17
July	68.4	75	2.55
August... ..	69.7	71	2.74
September	69.7	73	6.36
October	70	75	6.86
November	70.7	74	1.27
December	70.3	74	6.62
January	69.8	76	2.15
February	71.9	73	1.99
March	70.4	77	4.61
April	69.9	77	5.62

Showing the distribution and incidence of infection of *Anopheles* in the selected localities in Kampala

Locality	Species of <i>Anopheles</i>	Total females caught	Stomach infected	Salivary glands infected	Double infection	Percentage infected
A.	<i>A. costalis</i> ...	962	10.5	11.7	1.7	20.5
	<i>A. funestus</i> ...	64	7.1	3.6	...	10.7
	<i>A. theileri</i> ...	66	10.6	10.6
B.	<i>A. costalis</i> ...	1,530	11.6	10.8	1.6	20.8
	<i>A. funestus</i> ...	140	18.8	5.3	2.6	21.5
	<i>A. theileri</i> ...	738	8.5	3.3	0.4	11.4
C.	<i>A. costalis</i> ...	426	11.4	13.2	0.8	23.8
	<i>A. funestus</i> ...	69	5	8.4	...	13.4
	<i>A. theileri</i> ...	64	9.6	9.6
D.	<i>A. costalis</i> ...	3,290	9.2	9.6	1.4	17.4
	<i>A. funestus</i> ...	5,388	8.4	9.2	1.4	16.2
	<i>A. theileri</i>
E.	<i>A. costalis</i> ...	1,211	9.6	9.1	1.3	17.4
	<i>A. funestus</i> ...	1,195	4.4	5.9	0.6	9.7
	<i>A. theileri</i>
F.	<i>A. costalis</i> ...	876	9.5	7.9	1.4	16
	<i>A. funestus</i> ...	259	5.5	4.3	...	9.8
	<i>A. theileri</i> ...	55	3.9	3.9

A CONDITION SUPERFICIALLY RESEMBLING MOSSY FOOT, IN A NATIVE OF THE SUDAN*

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PLATE I.

INTRODUCTORY REMARKS

This peculiar and believed hitherto undescribed appearance came to notice in the course of routine out-patient work in a provincial hospital located in a district of the Sudan where confidence in the strange medicine of the European has still ground to gain. In consequence, one still encounters from time to time unfamiliar medical conditions, particularly those characterised by chronicity and associated either with many years of simple neglect or, worse still, by periodic resort having been had to ignorant folk remedies.

In the Nuba Mountains area of Southern Kordofan, while the araboid peoples of the plains and the townees of mixed stock turn readily to local Government medical institutions for relief, the negroid Nuba tribes living comparatively remote in their hills are less sophisticated, and the sick do not always benefit by the medical aid which could be theirs for the asking.

It is indicative of progress that the subject of this note came voluntarily into hospital from the retirement of his hill, and not only asked for treatment but agreed to amputation when told that conservative measures would be futile. This shows great confidence in Government medicine, for the average Nuba is very loth to part with a limb. The history of the lesion went back thirty years.

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The absence of the sinuses so characteristic of mycetoma, which is a disease well-known locally, the length of the history and the superficial resemblance to mossy foot figured by Manson-Bahr (1925), led the writers to obtain what data could be collected with a view to establishing whether the condition was identical with or related to mossy foot, and, if neither, to place it on record as previously (as far as is known to us) undescribed.

THE CASE

Gamal Katna, a male Nuba aged about forty, of Nama village, Gulfan Moron, came to Dilling Hospital on December 18th, 1931 complaining of the offensiveness and uselessness of his left foot. This was much enlarged and misshapen, and obviously devoid of useful function. Nama village is situated on the side of a rocky hill. There is plenty of vegetation about, and there are also running streams. Bilharzia is endemic, and in the wet season malaria is a commonplace. The patient had not been out of the area in his life. His diet had always been that characteristic of his kind, millet, sesame, milk and occasional goat-flesh.

At the age of ten, that is, some thirty years previously, he had an ulcer (not a vesicle) on the anterior surface of his left leg immediately above the ankle joint. The ulcer had persisted ever since, at times apparently healing, only however to break out afresh later. Some fifteen years ago (fifteen years after initial lesion), he began to appreciate that the skin at the lower margin of the ulcer was thickening and becoming rough to the touch. Since then the affection of the skin had spread downwards and outwards, the roughness becoming more and more accentuated, and the foot becoming increasingly heavier and heavier, by reason, not only of a uniform enlargement, but also by the development of several bosses on the dorsum of the foot. About three years before (twenty-seven years after the initial lesion), the present condition of what might be termed maximum dermal involvement had been reached, and the ulcer had bared the tibia.

On admission the patient was emaciated and obviously weak. His temperature was 97.6° F. and his pulse rate was 120.

The lower third of the leg and the upper half of the dorsum of the foot were excavated by a moderately deep, indolent-looking ulcer weeping a serous discharge ; the margins were not undermined and were not notably lipped. The tibia was defined in the base of the excavation and appeared to have undergone a periosteal thickening. From the lower margin of the ulcer the foot exhibited, firstly a gross general enlargement, and secondly localised enlargements in the shape of bosses of various sizes projecting from the dorsum of the foot. The skin of the dorsum, the sides of the foot, the toes and the plantar surface showed a papillomatous growth which was absent from those areas of the sole and toes which would be in contact with the ground when walking was attempted. (The patient was able to distribute his body weight when in a standing posture, but walking in the sense of efficient pedal progression was not possible.) The individual papillae were anything up to 5 mm. in length, the longer ones being on the lower parts of the sides of the foot. These papillae were dry and horny, slightly tender to the touch, and when avulsed occasioned pain but no bleeding. The skin over the tendo Achilles was not involved. On certain portions of the dorsum it appeared that the papillae had been rubbed off, leaving a shiny reddish integument beneath. In the papillomatous areas tactile sensation was diminished ; sensation to pain was rather increased. The lower lip of the ulcer itself bore no growth. As far as could be appreciated, the bones of the foot were unaffected. There were no varicose veins to be seen and no lymphatics could be felt. There was no wasting of the leg or thigh muscles. The inguinal glands of both sides and the left femoral gland were enlarged, hard, fixed and slightly tender.

Examination of the heart revealed a soft haemic murmur. The arteries were definitely hard to compress. Examination of the lungs revealed no abnormality. The tendon and pupillary reflexes were normal. The fingers were clubbed, and the nails of the fingers were markedly convex. Palpation of the abdomen revealed a splenic enlargement of two fingers below the costal margin ; the liver appeared to be normal. The urine was tested : it was acid, had a specific gravity of 1017 and contained no albumen or sugar ; microscopically it showed some slight nondescript debris.

The foot and lower leg were removed on January 3rd, 1932. At

the operation the bone marrow was noted to be of unusual softness. After removal the affected area was incised, and it was noted that the actual integument was abnormally thin; it was, moreover, firmly adherent to the underlying tissue, which seemed to yield to the knife edge in a peculiarly easy fashion.

PATHOLOGIST'S REPORT

Dr. T. Hewar, of the Wellcome Tropical Research Laboratories, reported on the specimen as follows:—

The macroscopical appearance is that described and illustrated in Manson-Bahr, 1929, pp. 641-42. The chief features are the development of rough bosses and an astonishing hypertrophy of the papillae of the skin which project outwards as horny points. On sagittal section of the foot it is seen that the epithelium does not play much part in this process except where these gigantic papillae are present, and where ulceration has set in it is of normal appearance. Beneath the epithelium is a solid, opaque, whitish-grey layer about an inch in thickness overlying the deeper tissues. It is well defined and does not infiltrate the muscles or fascia beneath it. The bones and deep tissues generally appear normal. The dense white layer is very firm and fibrous and has blood vessels running through it but is otherwise devoid of any definite macroscopical structure.

Paraffin sections through numerous parts of the foot show a uniform change in the opaque white layer but otherwise nothing of note. The white material consists of a very dense acellular fibrous tissue with sleeve-like projections of granulation tissue surrounding all the small blood-vessels and the sweat glands. Bacterial stains of these sections show no organisms of any kind. There is nothing to give any clue to the aetiology. It appears to be a granulomatous condition with excessive deposit of fibrous tissue.

BACTERIOLOGY

Dr. E. Horgan, Government Bacteriologist, Wellcome Tropical Research Laboratories, examined a series of cultures from the blood and tissues of the affected region. He reported that no appearance of fungus was obtained, and all cultures (including anaerobic cultures) showed only a few colonies of *staphylococcus aureus*.

CONCLUSIONS

The condition differed from mycetoma fundamentally, in that there were no sinuses, no characteristic granules, no involvement of tendons and bones, no pitting of the tissues on pressure and finally no fungus was cultured ; further, patients with untreated mycetoma generally die in ten to twenty years.

The condition agreed with mossy foot in that the foot and ankle were involved, in that dense growths of painful papillae were present, except on the plantar walking surfaces, and in its general mossy appearance. It differed by having no history of an initial vesicle, by the papillae not being vascular, by no fungus being culturable, and by reason of its having occurred in a native of the Old World and not in an American.

It appears reasonable to ascribe the condition to the very long existent chronic irritation from the ulcer's discharges ; viewed mechanically, this would accord with the distribution of the lesions. Enquiries amongst Nubas, long resident officials and native medical staff failed to reveal the occurrence of similar cases, past or present.

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Cassell & Co., Lond.

EXPLANATION OF PLATE I.

Fig. 1. Showing ulcer, bosses and papillae.

Fig. 2. Sagittal section, showing dense fibrous tissue layer.



FIG. 1



FIG. 2

A STUDY OF THE HOUSE-HAUNTING CULICIDAE OCCURRING IN FREETOWN, SIERRA LEONE;

AND OF THE PART PLAYED BY THEM IN THE
TRANSMISSION OF CERTAIN TROPICAL
DISEASES, TOGETHER WITH OBSERVATIONS
ON THE RELATIONSHIP OF ANOPHELINES
TO HOUSING, AND THE EFFECTS OF ANTI-
LARVAL MEASURES IN FREETOWN

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The investigation described in the paper which follows was commenced in June, 1930, and completed in November, 1931, although observations regarding mosquito bionomics were continued to a later date. The work was carried out in Freetown and the neighbouring village of Kissy, and in addition some of the observations regarding the bionomics of anophelines were made in the Port Loko district of Sierra Leone.

Freetown, the capital of Sierra Leone, is a city of 55,359 inhabitants, the African population numbering 54,673, the Asiatic 401, and the European 285 (1931 census). It is in latitude $8^{\circ} 29'$ North and longitude $13^{\circ} 13'$ West, and lies between the sea and a range of hills which rise to 1,000 feet or more behind it. Between the town and these hills lies Tower Hill at an elevation of some 400 feet, on the upper slopes of which are European dwellings, and on the lower slopes of which native houses encroach. The climate is warm, but seldom rises above 90° or falls below 65° in the shade. It is very damp, except during those short periods in December and January when the dry Harmattan wind blows. There is one rainy season beginning in April or May and ending in October or November, with a maximum fall in July, August and September, the average annual rainfall for the period 1902-1931 being 140 inches.

The village of Kissy is a native settlement of some 2,000 inhabitants ; no Europeans are living in the district. It is situated on the sea coast some six miles south-east of Freetown, and lies on a gentle slope between the hills and an area of level ground which separates it from the sea. The same meteorological conditions prevail as in Freetown.

The work reported here was undertaken with the co-operation of the Government Medical Department, who assisted us in every way and supplied the necessary native sanitary inspectors and labourers. We take this opportunity of acknowledging our indebtedness to Dr. J. C. S. McDouall, Director of Medical and Sanitary Services, and to the other medical officers concerned ; also to Mr. O. G. Price, Sanitary Engineer to the Sierra Leone Government.

PART I

A SURVEY OF THE HOUSE-HAUNTING MOSQUITOES OCCURRING IN FREETOWN AND THE NEIGH- BOURING VILLAGE OF KISSY, TOGETHER WITH SOME OBSERVATIONS ON OUTDOOR BITING

I. INTRODUCTION

Ever since the discovery of the part played by mosquitoes in the transmission of tropical diseases, there has been a steady accumulation of information regarding the bionomics of the insects concerned, but, in so far as West Africa is concerned, the information accumulated is, with certain notable exceptions, almost entirely confined to a description of the number and nature of their breeding places. It is now generally recognised that, in order to utilise the information obtained from a larval survey, it is essential to correlate it with a study of the species and numbers of mosquitoes occurring in the houses and biting out of doors. The real value of a larval survey is to allow of immediate action being taken to get rid of the potential vectors of disease, as shown by the mosquitoes collected in the houses and biting out of doors. That such a larval survey does not necessarily indicate what is the most important vector in the district can be shown by many instances. To take two examples, Schwetz (1927), in Elizabethville in the Belgian Congo, found by larval

surveys that the most common mosquito was *Culex duttoni* (90 per cent. of all larvae collected), but in the examination of houses encountered it very rarely, whereas in the same houses *C. fatigans* and *A. costalis* abounded. Similarly Gordon and Macdonald (1930) in Sierra Leone found large numbers of *A. rhodesiensis* larvae in numerous breeding places within 150 yards of some houses, in which, out of 117 adults collected, only five proved to be *A. rhodesiensis*, the remainder being composed of *A. costalis*, whose breeding places were much further away. We believe that the larval breeding places of mosquitoes in Freetown has been sufficiently dealt with by Blacklock and Evans (1926) and the various reports issued by the Government Medical Department. Previous observations, however, on the numbers and species of the mosquitoes occurring in houses in Sierra Leone, which have been made by various writers, such as Stephens and Christophers (1900), Wood (1915), Blacklock (1925), Gordon (1928), Gordon and Macdonald (1930), only deal with a small number of mosquitoes which were not collected in a systematic manner. In view of the lack of accurate information regarding this important subject, we decided to carry out a somewhat extensive examination of native houses in Freetown, and we later extended our survey to include the native village of Kissy.

The main object of the enquiry was, as stated in the title, to acquire information regarding the transmission of certain tropical diseases, especially malaria and filariasis, but it has also enabled us to make observations regarding the presence of potential yellow fever transmitting mosquitoes in the houses examined.

II. METHODS EMPLOYED IN SEARCHING THE HOUSES AND RECORDING OBSERVATIONS

In Freetown, during the rainy season (June to November inclusive) of 1930 and 1931, daily visits between the hours of 7 a.m. and 9 a.m. were made by one or more of the authors, assisted by a native inspector lent by the Sanitary Department. A number of different houses were thus visited daily, and those rooms which had been occupied during the previous night were searched for mosquitoes; during the period 3,587 rooms were examined. Our survey was greatly facilitated by the use of a large scale (1 : 1250)

map of Freetown which had recently been published. On this map even the smallest native huts were clearly marked, a point of great importance when conducting a search involving the examination and re-examination of large numbers of houses, many of which were unnumbered. The houses chosen were nearly all of the poorer class, small and dark, built of wood or mud, with a galvanised or thatched roof, as it was found that houses of the better class, which were light and well ventliated, did not often harbour mosquitoes, at any rate during daylight hours.

The object of our survey was to capture so far as possible every mosquito which existed in the room at the time of our visit. To achieve this result, all doors and windows were first closed, and then a very meticulous search of the room carried out by means of electric torches ; not only the walls but every article of furniture, including the under surface of the beds, was examined. The roof usually represented the most difficult problem, and for its search we were equipped with a step-ladder. The time occupied in thus searching a room naturally varied greatly with the size and type of room examined, but usually we found that two persons could complete the search of an average sized bedroom in from ten to fifteen minutes.

The native sanitary inspectors who assisted us proved extremely helpful and efficient, but owing to the necessity for sometimes changing these officers and for various other reasons, it was decided that the entire collecting of mosquitoes and recording of results should be done by the authors. We attach some importance to this fact as we do not consider that the results recorded by native collectors, no matter how carefully trained, can be regarded as entirely reliable. The data recorded as the result of inspection and subsequent dissection of the captured mosquitoes were limited to those which appeared to be of importance as regards the disease-carrying possibilities of the insect ; it has, however, been shown by various authors that the recording of certain observations, which at first might seem to have no bearing on the pathogenic importance of the insect, are frequently of great value in this respect. In making a selection of the points to be recorded, we have in the main followed the suggestions of Christophers, Sinton and Covell (1928). It will be noted, however, that we do not usually record the presence or type of blood meal found in the mosquitoes dissected. It was

unfortunate that this could not always be done, but we regard a mere statement as to the presence or absence of fresh blood as of little value without identification of the type of host from which the meal was obtained. As the majority of the mosquitoes had to be kept for forty-eight hours before dissection, we were unable to carry out this latter examination, and we have therefore omitted all such references.

On pages 279 and 280 is shown a copy of one of the forms used and our method of entering the observations. Some of the results recorded will be dealt with in the present paper, others in Parts II and III. It will be noted that the Culicini were identified in Liverpool, and we are indebted to Dr. Alwen M. Evans for undertaking this work. After identification in Liverpool, the culicines were returned to the laboratory in Africa so that a type collection was gradually formed. With the aid of this type collection and a key of the house-haunting culicines of the district, which Dr. Evans prepared, we were finally able to identify with certainty the species of Culicini captured. As a result of the earlier method of identification, it was not possible to carry out any dissection of the culicines captured, but, as we will show later, the number captured was so small that the results of dissection would probably have been almost valueless. We believe that our records of the species and their relative numbers occurring in the houses, considered together with the results of experimental infection with *W. bancrofti*, give a reliable idea of the part played by culicines in the transmission of filariasis in Sierra Leone.

III. RESULTS OBTAINED

I. *Species of Culicidae recorded.* The following species of Culicidae were collected from native houses in Freetown and Kissy during 1930 and 1931:

ANOPHELINI: *A. costalis* (*A. gambiae*), *A. funestus*, *A. nili*, *A. rhodesiensis*, *A. rufipes*. CULICINI: *C. annulioris*, *C. cinereus*, *C. annulioris* var. *consimilis*, *C. decens*, *C. decens* var. *invidiosus*, *C. duttoni*, *C. horridus*, *C. nebulosus*, *C. rima*, *C. thalassius*, *S. fasciata*, *T. (Mansonioides) africanus*, *L. tigripes* var. *fusca*, *Aedes (Aedimorphus)* sp.

DISTRICT Kissy (North) STREET Parsonage SHEET No. 5
 HOUSE No. 2a OWNER Williams DATE 17.x.30
 TYPE OF HOUSE Mud foundations
 Mud walls
 Thatch roof WIND E.S.E.
 TOTAL OCCUPANTS 9 ADULTS 6 CHILDREN 3
 REMARKS Total rooms = 3, all used for sleeping.
 Three rooms searched.
 All rooms small, dark, and badly kept.
 Suitability for mosquitoes = + + +

TOTAL ANOPHELINE 23 M 0 F 23
 TOTAL CULICINES 1 M 1 F 0

LIVERPOOL REPORT ON CULICINES. DATE SENT 20.xi.30 FILE No. 11/14

Species.	M.	F.	Total.	Species.	M.	F.	Total.
<i>C. nebulosa</i>	1		1				

SUMMARY OF ANOPHELINE DISSECTIONS.

Species.	Total Dissections.	Positive Glands.	Positive Stomach.	Positive Glands and Stomach.
<i>A. costalis</i>	4	1	0	0
<i>A. funestus</i>	19	2	1	0

NUMBER	DATE	SPECIES	WINGS	MALARIA.			OVARIES	ROSS'S SPORES	MICROFILARIA	FLAGELLATES	SPIROCHAETES	OTHER HELMINTHS	SLIDE	REMARKS
				GLANDS	STOMACH	PIGMENT								
1	19.x	Cost.	3				5							
2	"	"	3	+			5							
3	"	"	4				5		+					mf. = 1 proboscis, 4 thorax.
4	"	"	1				5							
5	"	Fun.	3				5			+			14	Stomach and Malpighian tubes.
6	"	"	2				5							
7	"	"	3				5							
8	"	"	2				2							
9	"	"	2				5		+					8 sausage forms thorax.
10	"	"	1				5							
11	"	"	2				5							
12	"	"	3		+†	+‡	5							† = 5 oöcysts, 1 mature. ‡ = some brown, some black.
13	"	"	4				5+							
14	"	"	3				5							
15	"	"	2				5							
16	"	"	2	+			5							
17	"	"	3				?							Diseased ovaries, ? nematode ova.
18	"	"	3				5							
19	"	"	2	+			5				+			*Scanty in glands. Short form with four spirals.
20	"	"	1				2							
21	"	"	3				5							
22	"	"	2				5					+	15	Free in abdominal haemocoel length = 1.5 cm.
23	"	"	3				5							

The above entries have been selected from various dissection sheets.

The annual medical and sanitary reports of various West African Colonies, and articles published by various observers, especially Johnson (1919), have supplied sufficient data to enable us to state that this list of house-haunting mosquitoes agrees closely with similar lists made in Nigeria and the Gold Coast.

2. *The numbers and proportions of Anophelini and of Culicini occurring in Freetown and in Kissy.* We have already pointed out that the mosquito survey of Freetown was later extended to include the native village of Kissy. The observations in Freetown were only made during the rains, those in Kissy during the dry and rainy seasons. In order, therefore, to compare the relative numbers of culicines and of anophelines occurring in these two districts, it is necessary to consider only those figures which deal with the same seasonal period ; this has been done in Table I.

TABLE I

Showing the number of sleeping rooms searched and the number of Culicini and Anophelini found per room, amongst a total of 3,005 rooms examined in Freetown during the rainy seasons of 1930 and 1931, and amongst a total of 358 rooms examined in Kissy during a similar period.

District	Number of rooms examined	Culicini		Anophelini		Ratio of Culicini to Anophelini
		Number caught	Number per room	Number caught	Number per room	
Freetown ...	3,005	82	0.027	1,223	0.406	1 : 15
Kissy ...	358	46	0.128	3,763	10.51	1 : 82

Table I proves that both the culicine and anopheline concentration is far greater in Kissy than in Freetown, being in the former case five times as great, and in the latter case twenty-six times as great. The reasons for this difference will be discussed in Part III under the results of anti-mosquito measures. The comparative paucity of culicines as compared with anophelines in the houses both in Freetown and Kissy is very striking ; it also appears to vary considerably in different colonies and in different districts of the same colony. Unfortunately, figures dealing with this subject are not numerous. Below, in Table II, we give such references as are available.

TABLE II

Showing the ratio of house-haunting Culicini to Anophelini, as noted by various observers in West African Colonies.

Colony	District	Authority and date	Total number of Culicini found	Total number of Anophelini found	Ratio of Culicini to Anophelini
Gold Coast ...	Takoradi ...	Pomeroy (1931) ...	357	2,195	1 : 6
Gold Coast ...	Accra ...	Macfie (1922) ...	276	10	28 : 1*
Nigeria ...	Lagos and Ikoyi	Connal (1930) ...	350	295	1 : 1
Nigeria ...	Kaduna ...	Johnson (1919) ...	902	4,541	1 : 5
Nigeria ...	Katagum ...	Johnson (1919) ...	15	3,995	1 : 266
Nigeria ...	Zungeru ...	Johnson (1919) ...	382	1,679	1 : 4
Nigeria ...	Gadua ...	Taylor (1930) ...	74	2,134	1 : 29
Sierra Leone ...	Kaballa ...	Wood (1915) ...	75	478	1 : 6
Sierra Leone ...	Freetown ...	Gordon <i>et al.</i> (1932)	82	1,223	1 : 15
Sierra Leone ...	Kissy ...	Gordon <i>et al.</i> (1932)	46	3,763	1 : 82

* Macfie points out that this figure is misleading, 80 per cent. of the total culicines having been taken in a single house during the same month by the same collector.

If we omit the Accra results, which for the reasons given in the footnote appear misleading, we find that there seems to be a general tendency in all parts of West Africa for anophelines to predominate over culicines in the houses examined.* Johnson (1919) has drawn attention to the fact that a 'larval survey' of a district may, and probably will, give an entirely erroneous idea of the domestic mosquitoes likely to be found in the houses. We are entirely in agreement with his remarks; thus a larval survey of Freetown and Kissy would certainly show a great preponderance of culicines over anophelines, whereas the reverse is actually the case as regards the numbers of adults found in the houses.

In Table I we show the culicine concentration per room in Freetown and Kissy to be 0.03 and 0.13 respectively. The West

* Although exceptional cases are recorded; thus Connal (1924) notes that at Yaba (Lagos) *T. (Mansonioides) africanus* greatly outnumbered all the other mosquitoes caught in native houses. We have not included this observation in Table II, as he gives no figures.

African Yellow Fever Commission has shown that the concentration in Lagos is 1.79 during the wet season, a figure based on the examination of 368 rooms.* We have failed to find any other references to the concentration of culicines in the houses in West Africa, a somewhat important omission, in view of the constant outbreaks of yellow fever in this part of the world.

As regards the anopheline concentration, very few figures are available dealing with West Africa in general, but the work of Barber and his colleagues (1931) has supplied us with adequate figures for Nigeria. Below, in Table III, we compare the results obtained in Sierra Leone and Nigeria. Owing to the great variations in the numbers of anophelines in the dry and the wet seasons, we have rearranged the Nigerian figures into these two groups.

It will be seen from Table III that the anopheline concentration is remarkably low in Freetown as compared with Lagos or Ibadan.

TABLE III

Showing the anopheline concentration in native houses during the wet and the dry seasons, as noted by observers in Nigeria and Sierra Leone.

Colony	District	Authority and date	Season	Number of rooms examined	Total anophelines found	Number of anophelines per room
Nigeria*	Lagos and suburbs	Barber and Olinger (1931)	Wet ...	2,321	14,608	6.3
			Dry ...	2,629	10,748	4.1
	Ibadan ...	Barber and Olinger (1931)	Wet ...	997	852	0.9
			Dry ...	401	752	1.9
Sierra Leone ...	Freetown	Gordon <i>et al.</i> (1932)	Wet ...	3,005	1,223	0.4
			Dry ...	60	6	...
	Kissy ...	Gordon <i>et al.</i> (1932)	Wet ...	358	3,763	10.5
			Dry ...	224	244	1.1

* The West African Yellow Fever Commission has supplied us with figures, already acknowledged in a footnote, showing the anopheline concentration in houses on the island of Lagos. These, unlike Barber's figures, show the low anopheline concentration of 0.1 in the wet season and 0.05 in the dry season. They have been omitted from consideration, as Barber's observations, though not all on the Island, conducted in the same city and at the same time of year, are more adequate.

* These as yet unpublished figures are quoted by the courtesy of the West African Yellow Fever Commission, International Health Division, Rockefeller Foundation, in Lagos, who supplied the information to the Nigerian Medical Department.

This is especially noteworthy when it is recalled that our observations were conducted during the wet season in the most malarious portion of the town, and that preference was given to searching the most likely houses. We give no adequate figures for mosquito concentration in Freetown during the dry season; the number of mosquitoes found in test searches was so small that, in order to obtain adequate figures, it would have been necessary to search far more than the 3,000 rooms examined in the wet season. We did not think that the results obtainable from thus prolonging the survey would have justified the extra time and labour involved. The number of anophelines to be found in native houses in Freetown during the dry season must be surprisingly small, the six anophelines recorded were only obtained by searching specially selected houses on the outskirts of the town, which in the wet season were known to harbour the greatest number of anophelines.

This difference in the anopheline concentration in Freetown and Lagos is reflected in the proportions of children found infected with malaria in the two cities. Thus, Barber and Olinger (1931) examined 4,749 children, by means of thick films, over a period of twelve months, and found that, in the age period birth to fifteen years inclusive, 67 per cent. were infected. Gordon and Davey (1932), using the same technique, examined similar age groups to those of Barber and Olinger, and found that, amongst 1,804 children examined in Freetown over a twelve months period, 44 per cent. were infected with malaria.

3. *The prevalence of certain species of Culicini and Anophelini in Freetown and in Kissy.* A total of 5,371 mosquitoes (141 culicines and 5,230 anophelines) were captured in Freetown and Kissy. Below, in Table IV, we show the numbers of the various species captured in the houses.

Table IV shows that *C. nebulosus* and *C. cinereus* were the commonest house-haunting culicines, and *A. costalis* and *A. funestus* the commonest anophelines encountered. It is now necessary to consider the relative numbers of the different species, captured respectively in Freetown and in Kissy, of (a) Culicini, (b) Anophelini.

(a) *Culicini.* It can in general be stated that in Freetown and Kissy the proportions of the species were found to be similar; in both localities *C. nebulosus* and *C. cinereus* predominated, and, if we

omit these two species, we are left with a total of only forty-eight Culicini, comprising some thirteen species. This figure is too small to allow of any comparison being drawn as regards the predominance of any one species in the two localities.

TABLE IV

Showing the species found and their numbers, amongst 5,370 mosquitoes collected in 3,587 rooms in Freetown and Kissy during 1930 and 1931.

Species	Culicini			Species	Anophelini		
	Males	Females	Total		Males	Females	Total
<i>C. annulioris</i>	1	0	1	<i>A. costalis</i> ...	165	2,831	2,996
<i>C. annulioris</i> var. <i>consimilis</i>	1	0	1	<i>A. funestus</i> ...	234	1,941	2,175
<i>C. cinereus</i>	6	35	41	<i>A. nili</i> ...	1	27	28
<i>C. decens</i>	7	8	15	<i>A. rhodesiensis</i> ...	11	19	30
<i>C. decens</i> var. <i>individuosus</i> ...	1	6	7	<i>A. rufipes</i> ...	1	0	1
<i>C. duttoni</i>	0	1	1
<i>C. horridus</i>	0	2	2
<i>C. nebulosus</i>	18	34	52
<i>C. rima</i>	0	1	1
<i>C. thalassius</i>	2	2	4
<i>T. (Mansonioides) africanus</i> ...	0	3	3
<i>L. tigripes</i> var. <i>fusca</i> ...	0	2	2
<i>Aedes (Aedimorphus)</i> sp. ...	0	1	1
<i>S. fasciata</i>	0	5	5
Unidentified for various reasons	4	1	5
Total	40	101	141	Total ...	412	4,818	5,230

One of the objects of the survey was to acquire information regarding the prevalence of potential yellow fever carrying mosquitoes in houses. Up to the present, only species of the genus *Aedes* and three other species of culicines, *Eretmopodites chrysogaster* and *T. (Mansonioides) africanus* and *Culex thalassius*, are known to be potential vectors of yellow fever in West Africa. No specimens of

E. chrysogaster, only six of *Aedes* and three of *M. africanus* were encountered in the houses examined in Freetown and Kissy, and we are therefore left with the somewhat surprising result that, amongst the 3,587 rooms examined, only a total of nine mosquitoes potentially capable of carrying yellow fever were captured; such a low figure calls for critical consideration. We have already mentioned that, with the exception of the Yellow Fever Commission's figures for Nigeria, information regarding the adult culicine concentration in houses in West Africa is scanty; but on the other hand figures are available regarding the proportion of the different species of Culicini encountered. In Table V we compare the proportions of house-haunting Culicini known to be potential vectors of yellow fever with those so far not incriminated, as noted by various observers in West African Colonies.

TABLE V

Showing the proportion of house-haunting Culicini known to be potential vectors of yellow fever to those so far not incriminated, as noted by various observers in West African Colonies.

Colony	District	Authority and date	Total number of potential vectors found	Total number of other Culicini found	Ratio of vectors to other Culicini
Gold Coast ...	Takoradi	Pomeroy (1931)	185	181	1 : 1
Gold Coast ...	Accra ...	Macfie (1921)	23	253	1 : 11
Nigeria ...	Lagos ...	West African Yellow Fever Commission (unpublished)	444*	3,836	1 : 9
Nigeria ...	Lagos and Ikoyi	Connal (1930)	18	332	1 : 18
Nigeria ...	Kaduna ...	Johnson (1919)	15	887	1 : 59
Nigeria ...	Katagum...	Johnson (1919)	2	13	1 : 6
Nigeria ...	Zunguru	Johnson (1919)	5	377	1 : 75
Sierra Leone	Freetown	Gordon <i>et al.</i> (1932) ...	4	76	1 : 19
Sierra Leone	Kissy ...	Gordon <i>et al.</i> (1932) ...	5	51	1 : 10

* Only refers to *Stegomyia fasciata* and *T. (Mansonioides) africanus*.

Table V shows that, with the exception of Takoradi, the proportion of potential yellow fever vectors which occur amongst the house-haunting Culicini is small. It is unfortunate that no figures regarding the actual number of potential yellow fever vectors

per room are available from most of the Colonies; the only references we are aware of are those, already referred to, from Lagos. If we compare our results with those obtained by the West African Yellow Fever Commission at Lagos, a remarkable difference is at once observable.

	Number of rooms searched	Number of potential yellow fever vectors found	Number of vectors per 1,000 rooms
Lagos... ..	368	139	380
Freetown	3,005	4	1.3
Kissy	582	5	8.6

How far these facts have any bearing on the absence of yellow fever outbreaks in Sierra Leone during recent years, we are not prepared to discuss.

Although the results so far recorded probably give some idea of the number of culicines which enter houses, it is extremely doubtful whether these observations give a true estimate of either the species or numbers of culicines which have entered the house during the previous twenty-four hours. It is a common observation in European houses in Freetown that culicine mosquitoes frequently enter the houses in very large numbers after dark, but few if any are to be found after daylight the following morning. On one occasion in a European house, we estimated the numbers in three rooms to be about 350. On the following morning at seven o'clock a search of the same rooms only produced nine culicines.

(b) *Anophelini*. We have already shown that five species of anophelines were captured during the survey; below, in Table VI, we show the concentration of these different species in Freetown and in Kissy.

Table VI proves that only one species of anopheline, *A. costalis*, occurred in appreciable numbers in the houses in Freetown, whereas in Kissy *A. costalis* and *A. funestus* were about equally represented, *A. nili* and *A. rhodesiensis* also being present, although in very small numbers. In Table VII we compare the incidence of the different species captured in Freetown and Kissy with the results obtained by workers in other parts of West Africa.

TABLE VI

Showing the concentration of certain species of house-haunting anophelines amongst a total of 1,223 captured in Freetown, and 4,007 captured in Kissy.

Species	FREETOWN		KISSY	
	Number of rooms examined, 3,005		Number of rooms examined, 582	
	Total number found	Number per room	Total number found	Number per room
<i>A. costalis</i>	1,214	0.4	1,782	3.06
<i>A. funestus</i>	9	...	2,166	3.72
<i>A. rhodesiensis</i>	0	...	30	...
<i>A. nili</i>	0	...	28	...
<i>A. rufipes</i>	0	...	1	...

It will be seen from Table VII that, in all the West African colonies from which records are available, *A. costalis* and *A. funestus* are by far the most common house-haunting anophelines, the other species, although widespread in their distribution, not occurring in sufficient numbers to be of great importance.

(4) *Outdoor biting experiments in (a) Freetown and (b) Kissy.*

(a) *Freetown.* We have shown that in Freetown there was an extremely low concentration of both culicines and anophelines in native houses when these were searched in the early morning. In view of the fact that the comparatively small number of mosquitoes in houses is associated with a high malaria infection rate (44 per cent.) amongst the children, we thought it advisable to make some observations on the species and numbers of mosquitoes to be found biting out of doors.

We carried out two small series of observations, the first towards the end of the rainy season, when anophelines were still relatively numerous in houses, and the second towards the end of the dry season and during the first part of the rains, at which time culicines are at their maximum concentration in houses. Native boys, varying in number from two to eleven, were employed on a series of nights between the hours of seven and ten. They were supplied with

TABLE VII

Showing the incidence of certain species of house-haunting anophelines as noted by various observers in different West African Colonies. ('N' indicates a negligible proportion, i.e. under 1 per cent.)

Colony	District	Authority and date	Total number of anophelines found	PERCENTAGE OF TOTAL ANOPHELES															
				<i>A. pretoriensis</i>	<i>A. obscurus (umbrosus)</i>	<i>A. costalis</i>	<i>A. funestus</i>	<i>A. nili</i>	<i>A. rhodesiensis</i>	<i>A. rufipes</i>	<i>A. pharoensis</i>	<i>A. obscurus</i>	<i>A. squamosus</i>	<i>A. mauritanus</i>	<i>A. flavicosta</i>	<i>A. hargreavesi</i>	<i>A. donicolus</i>	<i>A. theileri</i>	<i>A. moucheti</i>
GOLD COAST	Takoradi ...	Pomeroy (1932)	2,195	98	N	N	N
	Accra ...	Macfie (1921) ...	10	80	20
NIGERIA	Kaduna ...	Johnson (1919)...	4,541	N	...	81	17	N	...	1	N	...	N
	Katagum ...	„ (1919)...	3,995	25	67	7	1	...	N	N	N
	Zungeru ...	„ (1919)...	1,679	2	...	58	31	14	N	1	...	N
	Lagos and Ikoyi	Connal (1929) ...	295	...	2	95	3
	Lagos and vicinity	Barber and Olinger (1931)	15,727	96	N	2	N	...	N	...	1	N
	Lagos City	Barber and Olinger (1931)*	100
	Gadua ...	Taylor (1930) ...	2,134	47	44	N	...	1	2	...	4	2
	Ibadan ...	Barber and Olinger (1931)	1,104	68	28	4
LIBERIA	Firestone Plantation	Barber, Rice and Brown† ...	12,076	46	51	3	1	...
	Freetown ...	Ross, Annet and Austen (1900)	200 (approx.)	100
	Freetown ...	Gordon <i>et al.</i> (1932)	1,223	99	1
	Kissy ...	Gordon <i>et al.</i> (1932)	4,007	44	54	1	1	N

* We understand from Dr. Barber (private communication) that all the anophelines captured in houses in Lagos were *A. costalis*.

† Not yet published; a private communication.

test tubes and stationed at various suitable points in the town, and a small reward was offered for each mosquito captured in the act of biting, only those containing blood being accepted. The results are shown in Table VIII.

TABLE VIII

Showing the species and numbers of mosquitoes caught biting out of doors in Freetown in the evening during two seasons of the year.

Season	Month	Number of evening observations	Total number of 'boy hours' spent collecting*	Species and numbers of mosquitoes caught biting
Close of rains ...	Sept. ...	9	95	Nil
End of dry season and commencement of rains	April ...	5	83	<i>Aedes (Stegomyia) vittata</i> 2 <i>C. thalassius</i> 3
	May ...	22	322	<i>Aedes (Stegomyia) vittata</i> 7 <i>C. thalassius</i> 31 <i>T. (Mansonioides) uniformis</i> 1
	June and early July	20	63	<i>C. thalassius</i> 1 <i>C. (Culiciomyia) cinereus</i> 1 <i>A. costalis</i> 2 <i>A. theileri</i> 1
TOTAL ...		56	563	49

*The number of 'boy hours' spent at a single observation is obtained by multiplying the number of boys employed by the total time spent.

It is unfortunate that the number of observations and the time spent in collecting were not sufficient to allow us accurately to compare them with our much more extensive observations made in houses; certain interesting facts, however, are recorded in Table VIII. It will be seen that the species and their numbers found biting out of doors are different from those found resting in houses. *C. (Culiciomyia) nebulosus*, which represents one-third of the culicines found in houses, was never taken out of doors, an observation in agreement with that of Davis and Philip (1931) in Lagos, who, amongst fifty-four of the species examined by means of the precipitin test, found no evidence of human blood. On the other hand, *C. thalassius*, which was comparatively rare in houses, represented over half the culicines found biting out of doors. Little can be said about the small

number of anophelines captured; but the fact that only three were taken in a total of over 500 hours spent collecting supports our finding of a low anopheline concentration in houses. Perhaps the most important point adduceable from these evening catches is that, although the actual number of culicines captured was small and therefore agrees with our house observations, the proportion of potential yellow fever vectors caught biting is large. It will be seen that ten potential vectors (a proportion of one in five culicines) were caught during the fifty nights on which the experiments were carried out, whereas our total figures for house-haunting mosquitoes in Freetown and Kissy show that, out of 3,587 rooms examined, only nine potential carriers of yellow fever were found amongst the 141 culicines (a proportion of one in fifteen) captured.

(b) *Kissy*. Our experiments at Kissy were conducted at the beginning of the rainy season, when ten observations, totalling 119 boy hours, yielded nineteen Culicini and twenty-one Anophelini. We have shown that, at this season (June and early July) in Freetown during twenty observations, only two culicines and three anophelines were taken during sixty-three boy hours. Thus, the mosquitoes per boy hour in Kissy were four times as great as those taken in Freetown at the same time of the year. The following species and numbers of Culicini and Anophelini were captured in the act of biting, and all found to contain blood:—

<i>Culicini</i>				<i>Anophelini</i>			
<i>Aedes</i> (<i>Stegomyia</i>) <i>fasciata</i>	1	<i>Anopheles</i> <i>theileri</i>	11
„ „ <i>africana</i>	2	„ <i>mauritanus</i>	6
„ „ <i>vittata</i>	7	„ <i>costalis</i>	2
„ „ <i>luteocephala</i>	2	„ <i>funestus</i>	1
<i>Culex</i> <i>decens</i> var. <i>invidiosus</i>	1	„ <i>rufipes</i>	1
„ <i>thalassius</i>	3				
<i>Taeniorhynchus</i> (<i>Mansonioides</i>) <i>uniformis</i>			3				

So far as the Culicini are concerned, the remarks regarding outdoor biting in Freetown appear to be equally applicable to Kissy, though the proportion of potential yellow fever vectors captured in the latter was even higher. The anopheline results, so far as they can be judged from such small figures, are interesting, for they show that the two predominant species of anophelines found biting out of doors were *A. theileri* and *A. mauritanus*, yet

not a single specimen of either species was present amongst the 4,000 anophelines captured in houses in the same district. Barber and Olinger (1931) note that, amongst 517 anophelines caught biting out of doors in Lagos, 56 per cent. were *A. mauritanus*, a species of which only two were present amongst some 15,000 anophelines caught indoors. Again, *A. theileri*, which was the commonest mosquito noted biting out of doors at Kissy, is apparently almost unknown in houses throughout West Africa (see Table VII).

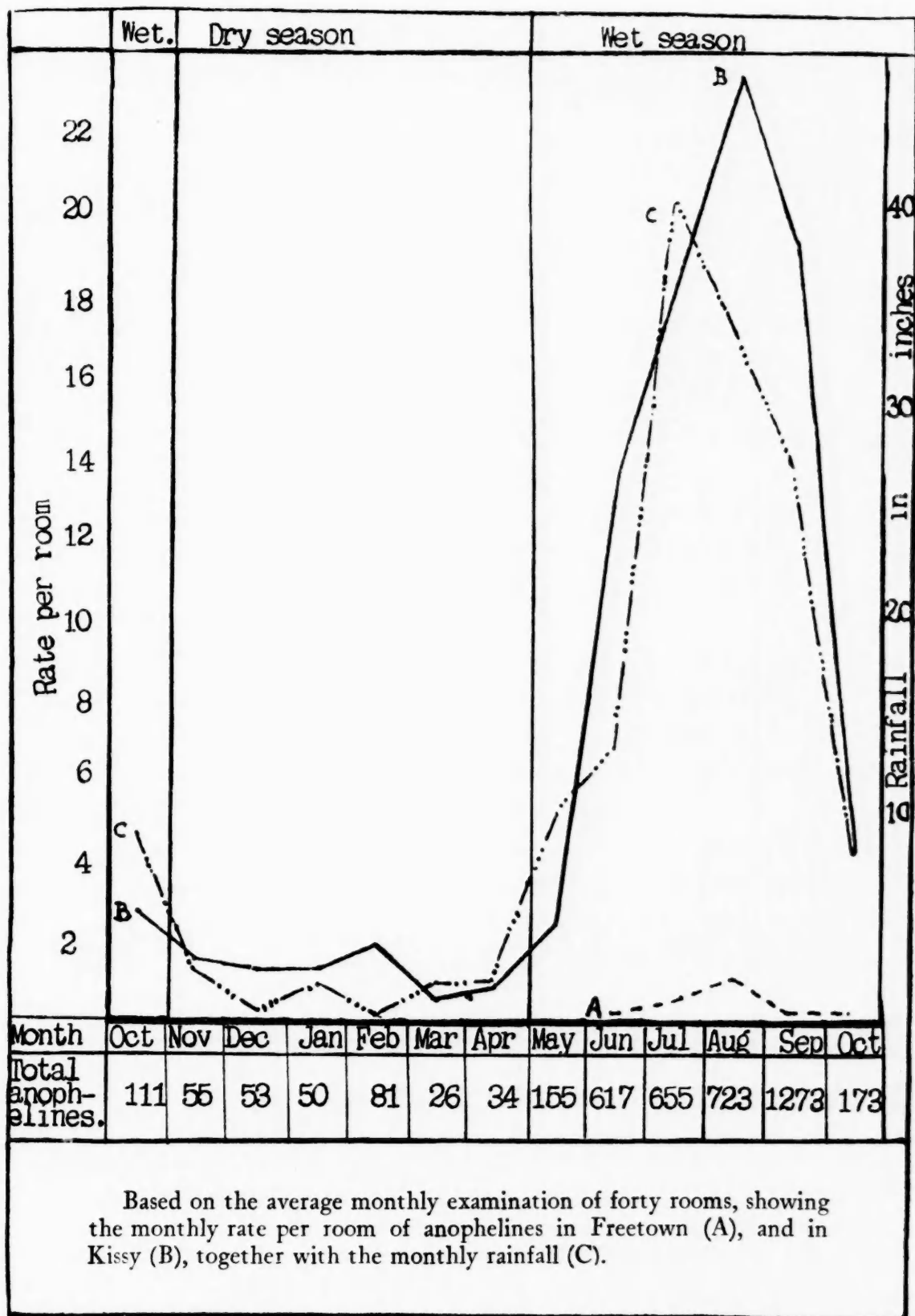
5. *The monthly incidence of Culicini and Anophelini in Freetown and in Kissy.* The total number of Culicini caught was too small to allow us to estimate their monthly incidence; it can only be stated that they reached their maximum concentration in the earlier part of the wet season. The monthly anopheline rates in Freetown and Kissy showed a marked relation to the rainfall, and are depicted in Graph I.

Graph I shows in a striking manner the relationship between rainfall and anopheline incidence both in Freetown and Kissy, and shows that the highest monthly concentration in Freetown (1 per room) during the rains fell far short of even the lowest monthly concentration in Kissy during the same period (3 per room in May). It is of interest to compare this maximum figure of one per room in Freetown with Barber's (1931) figure of fifteen per room in Lagos and its vicinity.

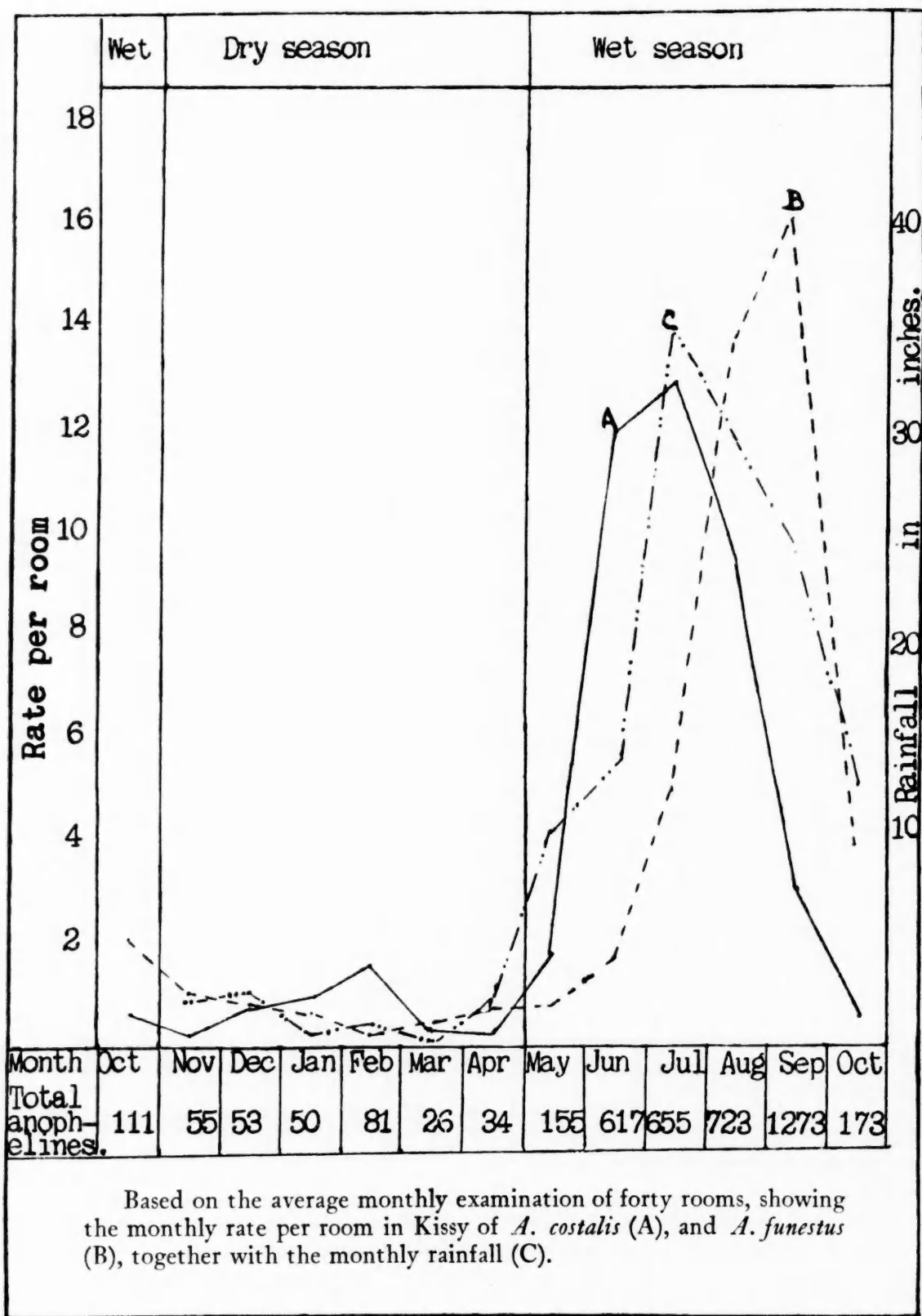
The monthly incidence of *A. costalis* and of *A. funestus* follow similar courses, but they are not superimposed, the *A. funestus* curve rising and falling in a similar manner to that of *A. costalis* but lagging some two months behind it, as is shown in Graph II. The few *A. nili* captured were all obtained in May and June, and the thirty *A. rhodesiensis* recorded were taken in July, August and September.

Graph II shows that, whereas the *A. costalis* rate begins to rise immediately after the first rains, and continues to rise until it reaches its maximum of thirteen per room in July, *A. funestus* only begins to rise in July, the month of maximum rainfall, and does not reach its highest point of sixteen per room until September. From this time it falls very sharply to four per room in October, while *A. costalis* falls more gradually to less than one per room in the same month. A similar observation has already been made in Nigeria by Taylor (1930).

GRAPH I



GRAPH II



IV. SUMMARY OF PART I

1. The species of house-haunting Culicini and Anophelini occurring in Freetown and the neighbouring village of Kissy are similar to those described as occurring in other West African Colonies.

2. In the native houses, in both Freetown and Kissy, there is a great preponderance of anophelines over culicines ; this appears to be usual throughout West Africa.

3. There is a great difference in the concentration of anophelines per room in the city of Freetown and in the adjacent native village of Kissy. But whereas the village of Kissy presents a similar state of affairs to other West African villages, Freetown has a far lower concentration of anophelines in the native houses than have similar large towns in Nigeria, there being only two from which figures are available.

4. The culicine concentration in Freetown and Kissy is very low in the native houses at the hours during which they were examined. Similar houses examined at similar hours in Nigeria contain far more culicines

The proportion of potential yellow fever carrying Culicini to non-vectors found in native houses in Freetown is similar to that found in other West African Colonies. But, owing to the general paucity of Culicini, the actual numbers found in the houses are less than those found at a similar hour in Lagos.

Evidence is produced which suggests that the Culicini caught in native houses in the early hours of the morning are not representative, either as regards species or numbers, of the total culicines which have entered the house during the previous twenty-four hours. This does not apply to anophelines, in which case the morning catch is probably representative of those which have entered the dwelling during the night.

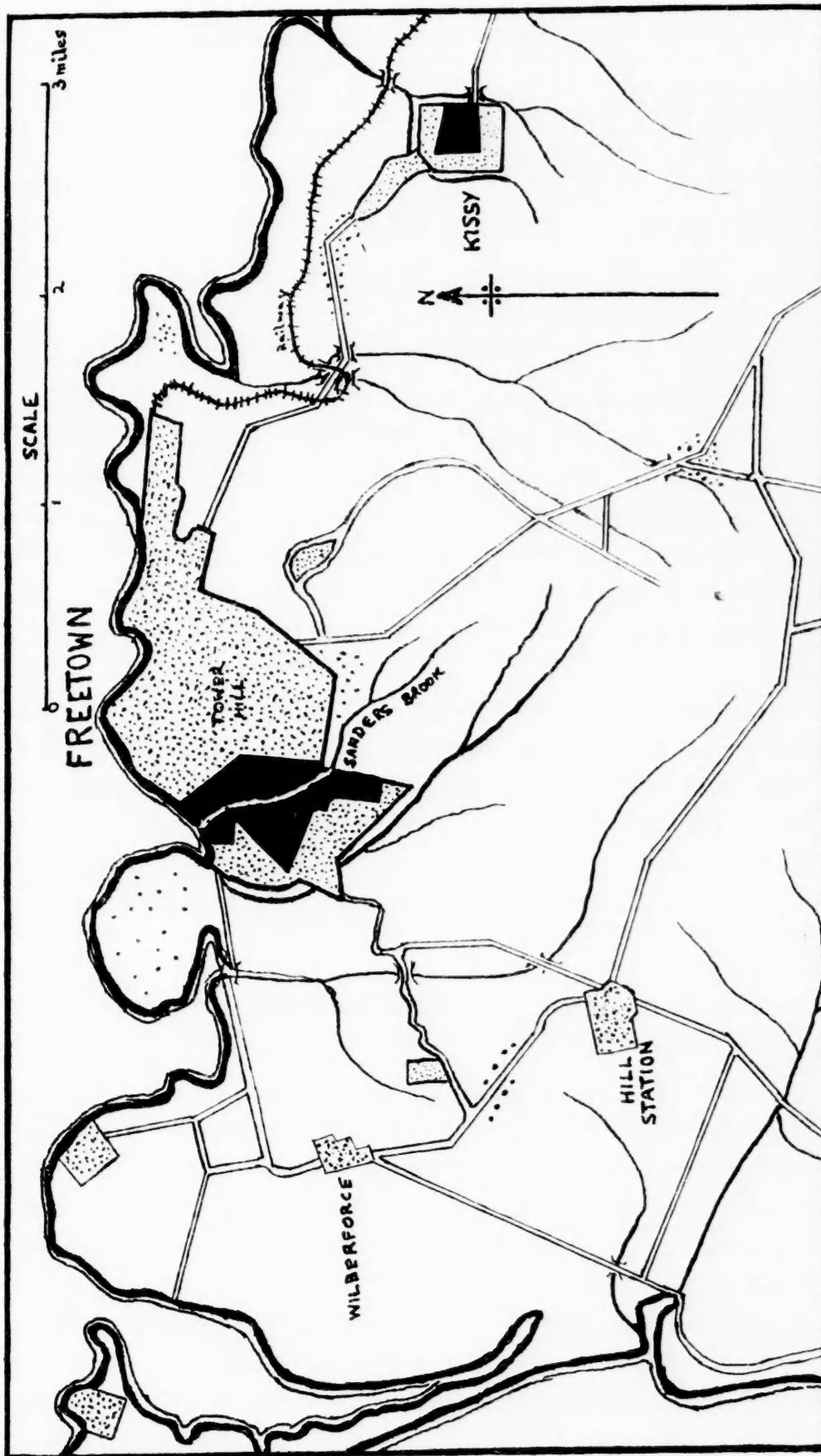
5. In Freetown, the only species of anopheline occurring in appreciable numbers in the houses is *A. costalis*. In Kissy, *A. costalis* and *A. funestus* appear to be of equal importance. Throughout West Africa these are the predominant house-haunting species.

6. A small number of outdoor biting experiments, conducted in Freetown during two seasons of the year, confirm the findings of house examination as regards the comparative paucity of mosquitoes

in Freetown, but show that a greater proportion of potential yellow fever vectors are taken biting out of doors than are found in houses in the early morning.

Similar experiments carried out in Kissy during the rains show that the mosquitoes found biting are four times as numerous as in Freetown, the proportion of potential yellow fever vectors amongst the Culicini being higher in the former. It is also noted that *A. theileri* and *A. mauritanus* were common outdoor biters, whereas not a single specimen of either species was taken indoors in the same locality, among 4,000 anophelines collected.

7. The monthly incidence of anophelines in Freetown and in Kissy shows, as might be expected, a very close association with the rainfall, and reaches its maximum at the height of the rains, this figure in Kissy being twenty-three per room, and in Freetown one per room. It was found that the rise in the *A. costalis* rate antedated that of *A. funestus* by some two months.



MAP I.—Diagram of Freetown and its suburbs, showing (in solid black) the areas surveyed in Freetown and in Kissy. The more populous districts are indicated by dotting. The area surveyed in Freetown is shown on a larger scale on Map II.

PART II

THE RESULTS OF DISSECTION FOR MALARIA AND
FILARIA OF HOUSE-HAUNTING ANOPHELINES
CAPTURED IN FREETOWN AND KISSY, TOGETHER
WITH OBSERVATIONS ON THE DISPERSION OF
ANOPHELINES FROM HOUSES

I. INTRODUCTION

In order to estimate the importance of any species of mosquito in the transmission of tropical disease, it is necessary, as far as possible, to establish the following points:—(1) To what extent the species in question bites man in houses or out of doors. (2) Whether, under experimental conditions, the mosquito in question will allow complete development of the parasite under consideration. (3) To what extent the species under investigation is found infected in nature.

We have already discussed in Part I the mosquitoes in Freetown and Kissy occurring in houses and biting out of doors, and have shown that the only two anophelines of numerical importance are *A. costalis* and *A. funestus*. It is now necessary to consider to what extent these species can be infected experimentally, and what proportion of them are found naturally infected with malaria and filaria.

II. EVIDENCE REGARDING THE EXPERIMENTAL INFECTION OF
A. COSTALIS AND *A. FUNESTUS* WITH (A) MALARIA AND (B) FILARIA

(a) *Malaria*. Evidence regarding the infectability of the two species in question with malaria rests on experiments carried out with *P. falciparum*. The species *P. vivax* was found to be too rare to supply sufficient donors, while in a previous paper (Gordon and Davey, 1932) we have already discussed our failure to infect either species with *P. malariae*. Gordon and Macdonald (1930) showed that *A. costalis* was capable of infection with *P. falciparum*, and, as shown in Table I below, this has been corroborated.

It is surprising that up to the present *A. funestus*, an anopheline which we have shown to be widespread in West Africa and closely

associated with man, has not been incriminated as a vector of malaria, except by the dissection of wild mosquitoes, a point already discussed in the paper referred to. Table IX records the results of feeding laboratory bred *A. costalis* and *A. funestus* on native children whose blood contained crescents.

TABLE IX

Showing the proportions of *A. costalis* and *A. funestus* which became infected after feeding on patients whose blood contained crescents.

Patient and number of feeding experiments	Crescents per 100 leucocytes	Species of anophelines fed	Number less than full fed	Number full fed	Number dissected ten or more days after feeding	Result : glands infected
N.W.	0.1-1.1	<i>A. costalis</i>	32	8	25	0
6		<i>A. funestus</i>	2	4	5	0
B.N.	1.8-40.0	<i>A. costalis</i>	4	4	5	2
3		<i>A. funestus</i>	7	22	23	0
J.M.	2.2	<i>A. costalis</i>	0	3	2	0
3		<i>A. funestus</i>	4	3	3	2
J.W.	16.1-47.2	<i>A. costalis</i>	0	1	1	0
3		<i>A. funestus</i>	0	8	7*	1

*One with oöcysts, in addition to the salivary gland infected mosquito recorded in the last column.

Table IX proves that *A. funestus* is capable of allowing the complete development of *P. falciparum*. The figures are not sufficiently large to enable us to state whether *A. costalis* or *A. funestus* is the more easily infected species, but the larger size of *A. costalis* is in its favour, as this insect probably takes up approximately twice as much blood as *A. funestus*, thus greatly increasing its chance of becoming infected. Again, *A. costalis* in our experiments bit man more readily than did *A. funestus*, as is shown by the following figures: of eighty-three *A. costalis*, fifty-two (63 per cent.) fed completely or partially, while of 113 *A. funestus*, applied under similar conditions to the same patients, fifty (44 per cent.) fed.

(b) *Filaria*. Four species of filariidae are known to occur amongst

natives in Freetown : *W. bancrofti*, *L. loa*, *D. perstans* and *O. volvulus*. Of these, *L. loa* is extremely rare throughout Sierra Leone and Chrysops is almost unknown in Freetown. Although *D. perstans* is widely distributed throughout the Colony and Protectorate, we have never encountered a case where the original infection was undoubtedly acquired in Freetown or its vicinity. *O. volvulus* appears to be limited in its distribution to the range of *S. damnosum*, which is unknown near Freetown. The remaining species, *W. bancrofti*, is widely distributed throughout the Colony and Protectorate of Sierra Leone. During the period of the survey, we examined the night blood of natives in hospitals and institutions in Freetown, and found that amongst the indigenous population about 8 per cent. were infected with *W. bancrofti*. One of us (Hicks, 1932), shows that *A. costalis* and *A. funestus* are capable of experimental infection with *W. bancrofti* as far as the infective stage, i.e., proboscis form.

These observations just quoted show that the two predominant house-haunting anophelines in West Africa, *A. costalis* and *A. funestus*, are capable of transmitting malaria and filariasis (*W. bancrofti*). It must be remembered that if sporozoites or filariae are found in wild anophelines, even if these, as in our experiments, are captured in houses or biting man in the open, they may have been derived from a non-human source. Although this is always a possibility, and the work of Green (1932) in Malay suggests that such a condition of affairs may give rise to confusion, we consider that it must be of rare occurrence throughout West Africa, and in the case of our observations in Freetown and Kissy need scarcely be considered, for in these neighbourhoods horses, cattle and pigs are but rarely encountered, the two former on account of trypanosomiasis and the latter being forbidden by law ; during the course of the survey we never saw or heard of monkeys in the areas examined. The remaining domestic animals (dogs, sheep, goats and fowls) are still a possible source of fallacy, but the work of Davis and Philip (1931) has demonstrated by means of precipitin tests that, although culicines captured in Nigeria gave a varying number of positive reactions with anti-sera other than human, *A. costalis* and *A. funestus* never did so. It follows therefore that the infections which we are about to describe as occurring in anophelines captured at Freetown and Kissy must have been derived from a human source.

III. TECHNIQUE OF DISSECTION, AND OBSERVATIONS RECORDED

In order to avoid recapitulation, we propose at this point to describe in detail our method of carrying out the examination of the captured mosquitoes, and to include in it certain points regarding the methods of estimating their probable age.

Each anopheline was caught in a tube, which was given a number referring to the house where captured, one record sheet being devoted to each house. They were then divided according to the houses, and each batch placed in a separate mosquito cage. This involved the use of a large number of cages, and we found the travelling cages described by Barraud (1929) very satisfactory for the purpose. The mosquitoes were supplied with water and kept for forty-eight hours before dissection, those dying within that period being placed in the ice chest and dissected as soon as possible after death. When the atmosphere was dry, it was found necessary to keep the cages continually moist by means of a slow overhead drip system, which allowed water to fall through the cage. At the end of the forty-eight hour period, the mosquitoes were collected, and each house collection dissected and recorded as a batch, chloroform vapour being used to kill them.

The dissection was carried out in the following stages :—

STAGE 1. The mosquito was placed on a slide, and the original diagnosis as to species was confirmed under the dissecting microscope. The wings were examined and placed in grades I, II, III or IV, according to Perry's classification (Perry, 1912).

STAGE 2. The wings and legs were removed and the head cut off with a sharp knife and placed in a drop of saline.

STAGE 3. A very small drop of saline was placed on the decapitated thorax, and using an 8 × 8 mm. coverslip the glands were gently squeezed out, as described by Barber (1930).

STAGE 4. The remainder of the body was transferred to a fresh drop of saline, and nicks made in either side of the penultimate abdominal segment. The ovaries and stomach were then withdrawn with fine forceps; these were separated and placed in saline under the small coverslips mentioned in the description of Stage 3.

STAGE 5. The severed head was steadied with one needle, while with the aid of another very fine needle, size 22, the proboscis was split from the base to the tip. We regard this careful splitting of the proboscis as a very important point. If it is simply cut across, filaria will sometimes be missed, whereas, if the technique is carried out as described, larvae in the proboscis will invariably be detected and will almost invariably emerge intact. The remainder of the head and body were then teased up, and placed under separate coverslips.

The dissection having been completed as described in Stages 1 to 5, the examination of the preparations was carried out under the compound microscope.

STAGE 6. The ovaries were examined and placed in age category 1, 2, 3, 4 or 5, according to Christophers' (1911) classification. The presence of a mature ovum in an otherwise immature ovary was regarded as evidence of previous ovulation, and the additional designation '5+' was used to describe this condition.

STAGE 7. The preparations were examined under the $\frac{1}{2}$ -inch objective for the presence of microfilariae, and note taken of the numbers present in the three regions, head, thorax and proboscis.

STAGE 8. The midgut was examined under the $\frac{1}{8}$ -inch objective for the presence of oöcysts, and if present their number and stage of development was recorded. Where pigment was present in the oöcyst, we paid particular attention to its examination, but we soon abandoned the attempt to classify it according to the species of plasmodium responsible for its production, because not only did we occasionally find all three forms of pigment present in the same mosquito, but sometimes there appeared to be more than one form of pigment in the same oöcyst. Judging by the few recorded successful infections with quartan malaria even so far as the oöcyst stage, the so-called 'typical quartan pigment' sometimes described as occurring in oöcysts must in most cases be regarded with suspicion.

The stomach was then ruptured and examined under the oil immersion for certain points to be noted later.

STAGE 9. The glands were examined under the oil immersion, and, having been crushed, the presence or absence of sporozoites was recorded.

It was our custom to carry out the dissection with a team of three workers, Stages 1 to 4 being allotted to the first worker, Stages 5 to 7 to the second, and Stages 8 and 9 to the third. By this arrangement, all members of the team could carry out their shares in approximately the same time. In all instances where further study of the specimen was required, as in the case of flagellates, spirochaetes, etc., preparations were made on a numbered slide, and if necessary stained. The dissection results obtained from each individual mosquito were recorded separately on the form.

IV. RESULTS OBTAINED FROM DISSECTION FOR (A) MALARIA, (B) FILARIA, OF ANOPHELINE CAPTURED IN HOUSES IN FREETOWN AND KISSY

We have already stated in Part I that only a few Culicini were collected in the houses, and that none of these was dissected. A total of 5,230 anophelines representing five species was collected from houses in Freetown and Kissy, of which 4,818 were females. Of these 3,267 were dissected in the complete manner just described.

In a certain number of instances it was impossible to carry out all of the examinations referred to, but these omissions were so trivial in number that in the Tables which follow we quote the actual correct percentages, without referring to the small number of

omissions which of necessity occurred. That is to say, if amongst 2,000 mosquitoes examined, 1,970 had the midgut examined and all the 2,000 had the glands examined, then the figures in the column under 'numbers examined' would be 2,000, but the percentage under the heading 'oöcysts' would be given as the proportion positive amongst 1,970. This trivial discrepancy is unavoidable whenever large numbers of mosquitoes are dissected.

(a) *Malaria*. Amongst a total of 3,267 female anophelines dissected from the collections made in Freetown and Kissy, 11.8 per cent. were found infected with malaria, but as we regard the percentage infected in the glands as the most reliable criterion, we have made use of this gland index when comparing together the infection rates of different species or in different localities. In Table X we show the proportion of the various species of anophelines infected with malaria in Freetown and in Kissy.

TABLE X

Showing the results of dissection for malaria and the sites of infection, amongst 1,164 anophelines captured in houses in Freetown, and 2,103 anophelines captured in houses in Kissy.

Species	FREETOWN				KISSY			
	Total examined	Percentage infected, all forms	Percentage infected in gut only	Percentage infected in glands	Total examined	Percentage infected, all forms	Percentage infected in gut only	Percentage infected in glands
<i>A. costalis</i> ...	1,156	11.1	3.2	8.0	1,157	16.3	5.2	11.2
<i>A. funestus</i> ...	8	12.5	12.5	0	908	7.0	2.9	4.1
<i>A. nili</i>	22	27.3	18.2	9.1
<i>A. rhodesiensis</i>	16	0	0	0
All species...	1,164	11.1	3.2	8.0	2,103	12.3	4.3	8.0

From these results it follows that *A. costalis*, *A. funestus* and *A. nili* all show a high infection rate in nature. As regards *A. nili*, although the infection rate is high amongst the small number dissected, the concentration of this species in Kissy is so very low (only twenty-eight captured amongst 600 rooms examined) that the part played by it locally in the transmission of malaria must be

insignificant in comparison with that of *A. costalis* and *A. funestus*. *A. rhodesiensis*, although capable of infection with malaria, would appear to play no local part in its transmission.

In Freetown, *A. costalis* represents all but a negligible proportion of the anophelines ; in view therefore of its extremely high sporozoite infection rate (8 per cent.), it must be held solely responsible for the transmission of malaria in Freetown. In Kissy, *A. costalis* and *A. funestus* are about equally represented and form 98 per cent. of the total anophelines captured. The latter shows a sporozoite rate of 4 per cent. and the former the remarkably high rate of 11 per cent. This higher infection rate of *A. costalis* is in part balanced by the numerical preponderance of *A. funestus*. Between them they share the onus of malaria transmission in Freetown and Kissy.

(b) *Filaria*. In malaria infection of mosquitoes, the presence of sporozoites in the glands is the usually accepted criterion of the infectivity of the insect. In filaria infection, however, there appears to be no such generally recognised standard, for it is difficult to define the particular stage of development of the filaria embryo in the mosquito, which should be accepted as evidence that further development up to the infective stage (proboscis form) may be regarded as reasonably certain. In view of the large number of species of mosquitoes which have been experimentally proved to be capable of allowing partial but not complete development of this parasite, it is obviously unreliable to accept the presence of 'gut and thoracic forms' as evidence of infectivity. On the other hand, to base the assumption only on the presence of proboscis forms has certain disadvantages, as it is recognised that their presence in this site, unlike the persistence of sporozoites in the salivary glands, only lasts for a short period. On the whole, we consider that the best criterion is that based on the presence of 'head and/or proboscis forms,' and we have, where possible, used this index in comparing together the infection rates in different localities, or amongst different species of mosquitoes.

In Table XI we show the percentage infected and the anatomical sites of infection with filaria amongst 3,267 anophelines captured in houses in Freetown and Kissy.

Table XI shows that the infection rate (head and/or proboscis forms) amongst anophelines captured in Freetown is 1 per cent., and

amongst those captured in Kissy 2 per cent. These figures, considering the short stay of the infective filaria larva in the intermediate host, must be considered high in Freetown and very high in Kissy. The fact that the infection rate amongst the *A. costalis* captured in Kissy is almost three times that in Freetown is very remarkable, especially in consideration of the close proximity of the two areas (see Map I), and that the difference in the malaria infection rate is not nearly so marked. *A. costalis* shows, just as it does in malaria, a far higher rate of infection than *A. funestus*; these

TABLE XI

Showing the results of dissection for filaria and the sites of infection amongst 1,164 anophelines captured in houses in Freetown, and 2,103 anophelines captured in houses in Kissy

Species	FREETOWN						KISSY					
	Total examined	PERCENTAGE POSITIVE IN					Total examined	PERCENTAGE POSITIVE IN				
		All forms	Thorax	Head	Proboscis	Head and/or proboscis		All forms	Thorax	Head	Proboscis	Head and/or proboscis
<i>A. costalis</i> ...	1,156	5.1	4.0	0.3	0.7	1.0	1,157	14.6	11.9	1.2	1.5	2.7
<i>A. funestus</i> ...	8	25.0	25.0	0	0	0	908	8.0	6.8	0.3	0.8	1.2
<i>A. nili</i>	22	4.5	4.5	0	0	0
<i>A. rhodesiensis</i>	16	0	0	0	0	0
All species...	1,164	5.2	4.2	0.3	0.7	1.0	2,103	11.5	9.5	0.8	1.0	2.0

figures, however, do not necessarily indicate the relative importance of the two species in Kissy as vectors of *W. bancrofti*, for the lower infection rate of *A. funestus* is compensated by the greater concentration of this species in houses. From the above figures, and from the data already given regarding experimental infection and anopheline concentration, we may conclude that, as in the transmission of malaria, *A. costalis* is the only anopheline transmitting filariasis in Freetown, and *A. costalis* and *A. funestus* the only anophelines of numerical importance transmitting it in Kissy.

It is probable that Culicini play some part in the transmission of *W. bancrofti* in Freetown and Kissy, for although the number

captured in houses in the morning is almost negligible in comparison with the anophelines, yet, as we have already shown, this figure probably falls short of the actual number of culicines which have entered the house during the night. Again, the number of culicines taken biting out of doors in Freetown, although relatively small, outnumber greatly the anophelines taken during the same period, though in Kissy they are about equally numerous. On the other hand, if we contrast the total number of known culicine vectors taken in houses and biting out of doors with the number of known anopheline vectors similarly captured, we must conclude that the part played by Culicini locally in the transmission of *W. bancrofti* is of negligible importance in comparison with that of the Anophelini.

V. THE MALARIA AND FILARIA INFECTION RATES AMONGST HOUSE-HAUNTING ANOPHELINES CAPTURED IN FREETOWN AND KISSY, COMPARED WITH THE RATES OBTAINED BY OTHER OBSERVERS IN VARIOUS WEST AFRICAN COLONIES

(a) *Malaria*. We have already shown in Table VII that throughout West Africa *A. costalis* and *A. funestus* are the predominant house-haunting anophelines. But in order to estimate their importance in the transmission of malaria it is also necessary to compare their infection rates with those of other and rarer domestic species. Thus, there are many instances to prove that the commonest house-haunting anopheline in the district is not necessarily the most important malaria vector: an excellent example of this is given by Strickland (1929), who in Assam caught and dissected 10,000 anophelines found in native huts. Amongst the three numerically prominent species thus captured, only a single infection was recorded, whereas *A. minimus* ('*funestus*'), which only came fourth in numbers (1,489 captured), accounted for no less than 94 per cent. of the total infections. Below, in Table XII, we summarise the literature regarding the infection rates amongst different species of anophelines occurring in the various West African Colonies.

Table XII shows that throughout the West African Colonies *A. costalis* and *A. funestus* have the highest sporozoite infection rate (with the exception of some inadequate figures for *A. nili*), and

TABLE XII

Showing the malaria infection rates amongst house-haunting anophelines in various West African Colonies, together with the percentage found infected in each species.

Colony	Locality	Authority and date	Species	Total examined	Percentage positive	Percentage with oöcysts	Percentage with sporozoites
GOLD COAST	Takoradi ...	Pomeroy (1931)	<i>A. costalis</i> ...	395	7	7	0
			<i>A. pharoensis</i> ...	10	0	0	0
			<i>A. funestus</i> ...	4	0	0	0
			All species ...	409	6.9	6.9	0
NIGERIA ...	Yaba ...	Connal (1924)	<i>A. costalis</i> ...	198	20	10.6	9.6
	Gadau ...	Taylor (1930)	<i>A. costalis</i> ...	1,936	17.6	10.0	7.4
			<i>A. funestus</i> ...	1,260	10.0	6.5	3.5
			<i>A. pharoensis</i> ...	205	0	0	0
			<i>A. squamosus</i> ...	83	0	0	0
			<i>A. mauritanus</i> ...	46	0	0	0
			<i>A. rufipes</i> ...	24	0	0	0
			<i>A. nili</i> ...	9	0	0	0
			All species ...	3,563	14.0	8.7	5.2
	Yaba ...	Barber and Olinger (1931)	<i>A. costalis</i> ...	6,453	11.8	7.2	4.6
	Lagos and vicinity	Barber and Olinger (1931)	<i>A. costalis</i> ...	15,144	12.0	5.5	6.6
			<i>A. funestus</i> ...	70	15.7	2.8	12.8
			<i>A. pharoensis</i> ...	281	1.8	1.1	0.7
			<i>A. mauritanus</i> ...	2	0	0	0
			<i>A. bargreavesi</i> ...	92	7.6	2.2	5.4
			<i>A. obscurus</i> ...	51	3.9	3.9	0
			<i>A. moucbeti</i> ...	87	2.3	1.1	1.1
			All species ...	15,727	11.7	5.4	6.4
	Ibadan ...	Barber and Olinger (1931)	<i>A. costalis</i> ...	753	21.6	7.9	13.1
			<i>A. funestus</i> ...	309	14.2	4.2	6.9
			<i>A. bargreavesi</i> ...	42	4.8	4.8	0
			All species ...	1,104	18.9	7.2	10.9
GAMBIA ...	Bathurst ...	Dutton (1903)	<i>A. costalis</i> ...	36	13.9	11.1	2.8
			<i>A. funestus</i> ...	24	8.3	4.2	4.2
			<i>A. pharoensis</i> ...	3	0	0	0
			All species ...	63	11.1	8.3	3.2
LIBERIA ...	Firestone Plantation	Barber, Rice and Brown	<i>A. costalis</i> ...	2,878	5.5	1.9	3.5
			<i>A. funestus</i> ...	2,698	4.6	2.7	1.9
			<i>A. theileri</i> ...	26	0	0	0
			<i>A. nili</i> ...	254	15.0	14.6	0.8
			All species ...	5,856	5.2	2.6	2.6

TABLE XII—continued

Colony	Locality	Authority and date	Species	Total examined	Percentage positive	Percentage with oöcysts	Percentage with sporozoites
BELGIAN CONGO	Stanleyville	Schwetz (1929)	<i>A. costalis</i> ...	992	19·8	8·4	11·5
			<i>A. funestus</i> ...	191	7·3	3·1	4·2
			<i>A. moucheti</i> ...	154	9·1	4·5	4·5
			<i>A. nili</i> ...	132	15·2	9·8	5·3
			All species ...	1,469	16·6	7·4	9·2
SIERRA LEONE	Freetown ...	Ross, Annett and Austen (1900)	<i>A. costalis</i> ...	109	25·0	19·3	5·5
	Koinadugu	Wood (1915)	<i>A. costalis</i> ...	91	15·0	7·2	8·7
			<i>A. funestus</i> ...	100	21·0	11·9	11·0
			<i>A. rhodesiensis</i> ...	37	2·7
			All species ...	228	15·8	9·6	10·0
	Freetown ...	Gordon et al. (1932)	<i>A. costalis</i> ...	1,156	11·1	3·2	8·0
			<i>A. funestus</i> ...	8	12·5	12·5	0
			All species ...	1,164	11·1	3·3	7·9
	Kissy ...	Gordon et al. (1932)	<i>A. costalis</i> ...	1,157	16·3	5·2	11·2
			<i>A. funestus</i> ...	908	7·0	2·9	4·1
			<i>A. nili</i> ...	22	27·3	18·2	9·1
			<i>A. rhodesiensis</i> ...	16	0	0	0
			All species...	2,103	12·3	4·3	8·0

that the total infection rate amongst all house-haunting anophelines collected in any Colony is represented by the infection rate of one or both of these species. It follows, therefore, that, as these two species are also the most numerous of the house-haunting anophelines, they must account for almost all the malaria in West Africa.

(b) *Filaria*. With the exception of Taylor's figures for Northern Nigeria and our own for Sierra Leone, there are few records of anopheline dissections for filaria sufficiently detailed to allow of discussion. Annett, Dutton and Todd in Southern Nigeria, so far back as 1901, showed that, of 281 *A. costalis* dissected, 5·7 per cent. contained filaria, and 0·7 per cent. were found infected in the head and/or proboscis. Connal (1924) at Yaba (Lagos) dissected some 200 *A. costalis*, and found 6 per cent. infected. Schwetz (1929), at Stanleyville in the Belgian Congo, dissected 1,000 *A. costalis*, and found 1·4 per cent. infected in the thorax, the infection rate

amongst the remaining species, *A. funestus*, *A. moucheti* and *A. nili*, being negligible. Below, in Table XIII, we compare our results in Sierra Leone with those obtained by Taylor in Nigeria, only the predominant species, *A. costalis* and *A. funestus*, being considered.

TABLE XIII

Showing the filaria infection rate, and sites of infection, in *A. costalis* and *A. funestus*, as noted by observers in Nigeria and Sierra Leone.

Locality	Gadau				Freetown				Kissy			
Authority and date	Taylor (1930)				Gordon <i>et al.</i> (1932)				Gordon <i>et al.</i> (1932)			
Species	Total examined	PERCENTAGE POSITIVE			Total examined	PERCENTAGE POSITIVE			Total examined	PERCENTAGE POSITIVE		
		All forms	Thorax	Head and/or proboscis		All forms	Thorax	Head and/or proboscis		All forms	Thorax	Head and/or proboscis
<i>A. costalis</i> ...	1,936	8.6	7.0	1.6	1,156	5.1	4.0	1.0	1,157	14.6	11.9	2.7
<i>A. funestus</i> ...	1,260	4.1	3.2	0.9	8	25.0	25.0	0	908	8.0	6.8	1.2
Combined species ...	3,196	6.7	5.5	1.3	1,167	5.2	4.1	1.0	2,081	11.7	9.6	2.1

Table XIII shows a high infection rate amongst *A. costalis* and *A. funestus* in Gadau, similar to that noted by us in Freetown and Kissy. Taylor, in addition to the figures given above, states that only in *A. costalis* and *A. funestus* were mature filaria larvae found, but that, in 5 per cent. of the 205 *A. pharoensis* and in 1.2 per cent. of the 83 *A. squamosus* dissected, immature infections (thoracic forms) were noted.

The scanty available evidence, which we have quoted regarding the part played by anophelines in the transmission of filariasis throughout West Africa, suggests that *A. costalis* and *A. funestus* are the only two species of importance.

VI. THE SEASONAL INCIDENCE OF MALARIA AND FILARIA INFECTION AMONGST ANOPHELINE CAPTURED IN FREETOWN AND KISSY

In Freetown, anophelines were too rare during the dry season (less than 0.02 per room) to allow of an adequate number being dissected. In Kissy, during the same season, their numbers were sufficient (1 per room) to allow us to estimate the infection rate for the whole season, but not sufficient to estimate the monthly incidence. During the wet season the number of anophelines in both places increased so greatly that we were able to determine their monthly infection rates.

(a) *Malaria*. Both *A. costalis* and *A. funestus* show very little seasonal variation in the sporozoite infection rate during the wet as compared with the dry season. The infection rate for *A. costalis* was found to be 10 per cent. in the dry season and 11.3 per cent. in the rains, while *A. funestus* maintained a constant infection rate of 4 per cent. throughout the year. In spite of the infection rate being the same in the wet and dry seasons, the risk of contracting malaria reached its maximum during the rains, because at this period the anopheline concentration in the houses was ten times as great as during the dry season. (See Table III, Part I.)

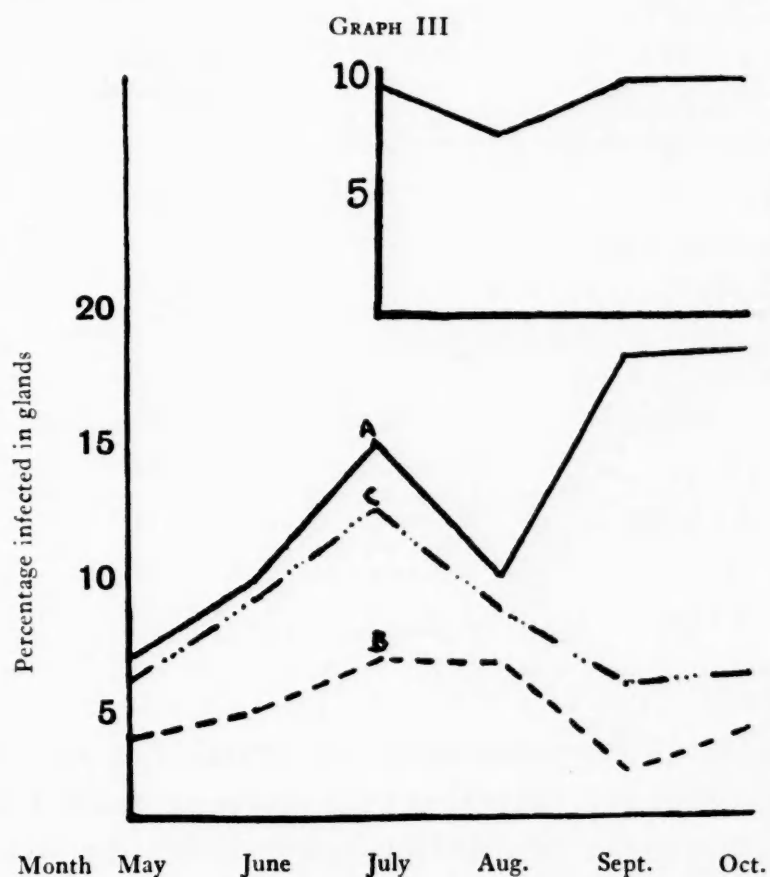
Although the infection rate in the rains is similar to that in the dry season, certain interesting fluctuations in the monthly incidence occur during the former period; these are depicted in Graph III.

Graph III shows that *A. costalis* and *A. funestus* do not reach their maximum infection rate during the same month. In the case of *A. funestus*, the peak is reached in July and August, and in *A. costalis*, in September and October. It will be noticed that there is a distinct decline in the infection rate of *A. costalis*, both in the Freetown and Kissy graphs, during the month of August, and that a similar drop occurs in the *A. funestus* infection rate in September. It is possible that this fall in the infection rate is due to a flooding of the infected anopheline population by newly emerged and therefore uninfected anophelines, for we have observed that the maximum incidence of *A. costalis* in houses occurs in June to July, whereas that of *A. funestus* occurs in July to August.

The total anopheline rate in Kissy shows very marked variations,

obviously due to the numerical preponderance of *A. costalis* in the first half, and of *A. funestus* in the second half, of the period.

(b) *Filaria*. Unlike the malaria infection rate, the filaria infection rate varied considerably according to season. In Table XIV we contrast the filaria infection rates of *A. costalis* and *A. funestus* in Kissy during the wet as compared with the dry season, and have included in the Table such meteorological data as might be concerned in their variation.



Showing the monthly sporozoite rate in Kissy during the wet season amongst *A. costalis* (A), and *A. funestus* (B). The sporozoite rate for all species is shown by the curve C.

Inset is the sporozoite rate in Freetown amongst *A. costalis* (the only species).

These figures show that the infection rate in the wet season is just three times as great as in the dry, the *A. costalis* rate being twice, and the *A. funestus* rate eight times, as great. This seasonal difference, which has also been recorded by Rao (1927) in India and by others, may depend on factors influencing the infection either in the human host or in the mosquito. Such evidence as is available regarding Freetown does not support the former view. Thus Young

(1914) examined the night blood of 858 persons, and found the same rate of infection (9 per cent.) in both the wet and dry seasons; Butler (1915) similarly examined 964 natives in Freetown, and found 11 per cent. infected in the dry season and 8 per cent. in the rains.

TABLE XIV

Showing the filaria infection rates as noted in the wet and in the dry season amongst 1,167 *A. costalis* and 908 *A. funestus* captured in Kissy.

			Dry season (8.6 inches rain)	Wet season (125.5 inches rain)
Relative humidity	66	80
Average maximum temperature	88	85
Average minimum temperature	75	74
Percentage infected, all forms	Both species	...	4.4	13.1
	<i>A. costalis</i>	...	8.5	15.5
	<i>A. funestus</i>	...	1.1	9.6
Percentage infected in head and/or proboscis	Both species	...	0.8	2.2
	<i>A. costalis</i>	...	1.5	2.9
	<i>A. funestus</i>	...	0	1.2

As regards factors influencing the infection in mosquitoes, it will be noted from the Table that little difference exists between the average temperatures recorded at Kissy during the wet and dry seasons, so that the well-known fact that low temperatures are prejudicial to developing filariae under experimental conditions (Bahr, 1912, and Fülleborn, 1908) would not appear to be the cause of the remarkable difference which we have recorded at Kissy. On the other hand, a very marked difference exists in the humidity during the two seasons, and it is possible that a high humidity may favour a high incidence of filaria infection amongst the anophelines.

VII. THE RELATIVE AGES OF THE ANOPHELINES CAPTURED IN FREETOWN AND KISSY, AND THE PROPORTIONS FOUND INFECTED IN THE VARIOUS AGE GROUPS

The relative ages of the anophelines captured in Freetown and Kissy. Perry (1912) has described a method of grouping anophelines in age periods according to the stage of deterioration of their wing scales. Christophers (1911) has similarly grouped them according to the stage of development of their ovaries; the two systems are considered together by Christophers, Sinton and Covell (1928). Throughout our work we have kept complete records by both classifications.

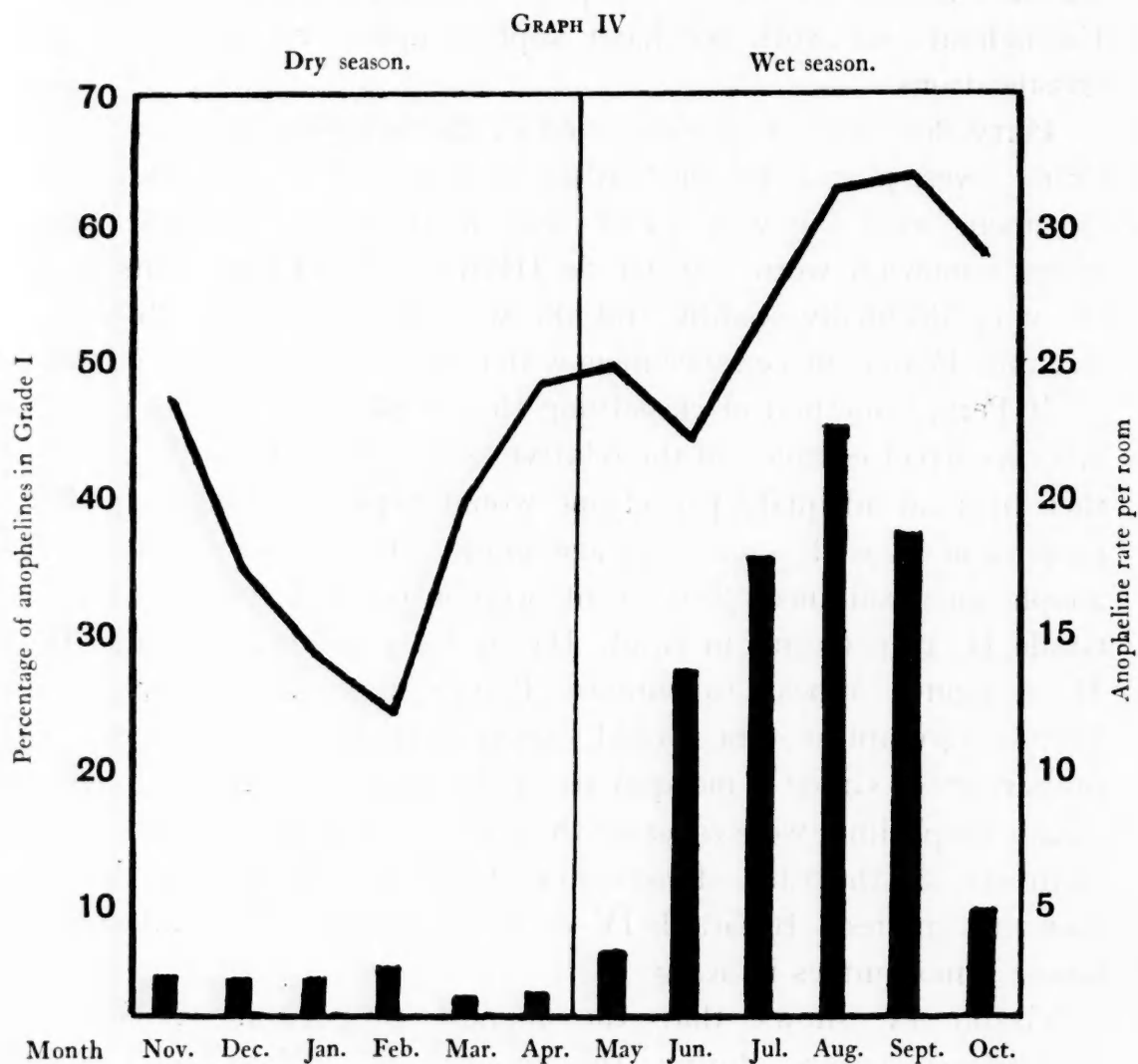
Perry describes his classification in the following manner: 'In Grade I were placed specimens with the wing well marked, and with specimens with the wing fairly well marked but with the wing fringe somewhat worn. In Grade III were placed specimens with the wing decidedly shabby and the wing fringe very much worn. In Grade IV were placed specimens with the wing actually threadbare.'

If Perry's method of classifying the anophelines in wing grades gives a correct estimate of the relative ages of those found in houses, then over an adequate period one would expect to find a smaller proportion in each successive age grade. In our series of 3,253 anophelines examined, 50 per cent. were in Grade I, 36 per cent. in Grade II, 12 per cent. in Grade III, and 2 per cent. in Grade IV. These figures appear to support Perry's theory. Moreover, on Perry's assumption, one would expect a marked increase in the proportion of Grade I mosquitoes in the houses at the time when young anophelines were entering them in the greatest numbers, that is to say, at the time of maximum breeding amongst the house-haunting species. In Graph IV we show the monthly incidence of Grade I mosquitoes in Kissy.

Graph IV shows that the highest proportion of Grade I anophelines occurs during the months of highest anopheline concentration, which is precisely what would be expected if Perry's Grade I mosquitoes do represent the youngest forms. The Graph also shows that, while the highest incidence of young anophelines occurs in August, September and October the lowest incidence occurs in December, January and February, when opportunities for breeding are at a minimum.

It is noteworthy that *A. costalis*, which has its maximum concentration in houses in May, June and July, shows the greatest incidence of young adults in April and May (with another rise in August). *A. funestus* has its highest concentration in houses in August and September, and shows the greatest incidence of young forms in July, August and September. It follows, therefore, that what is true of the total anophelines is also true of each species.

We have demonstrated that the increase in the proportion of



Showing the monthly incidence of Grade I (youngest) anophelines at Kissy amongst 2,064 examined; for the sake of comparison, the monthly anopheline incidence is also shown in vertical lines.

young anophelines, both in the combined and individual species, always precedes by a month or more the increase in the anopheline concentration in the houses, the former rise being disproportionately great. This phenomenon, so consistently present, may possibly be

explained on the assumption that the relatively large increase in the young forms is not sufficient to show an appreciable increase in the already existing large number of anophelines until a considerable accumulation has occurred. We believe we have proved by the foregoing statements that the monthly variations in the proportion of Grade I anophelines in the houses is best explained on Perry's assumption that they represent young anophelines, and that conversely Grades II, III and IV may be regarded as having survived for a longer period. In future, therefore, we propose to apply the term young anophelines to the former class, and old anophelines to the latter classes.

It is of interest to consider the incidence of old and of young anophelines in houses in Kissy throughout a twelve months period; this has been depicted in Graph V. This Graph is based on 4,006 anophelines caught in houses (see Graph I), of which 2,101 were actually examined as to wing grades, the ages of the remainder being estimated on the proportions found amongst the 2,101 examined.

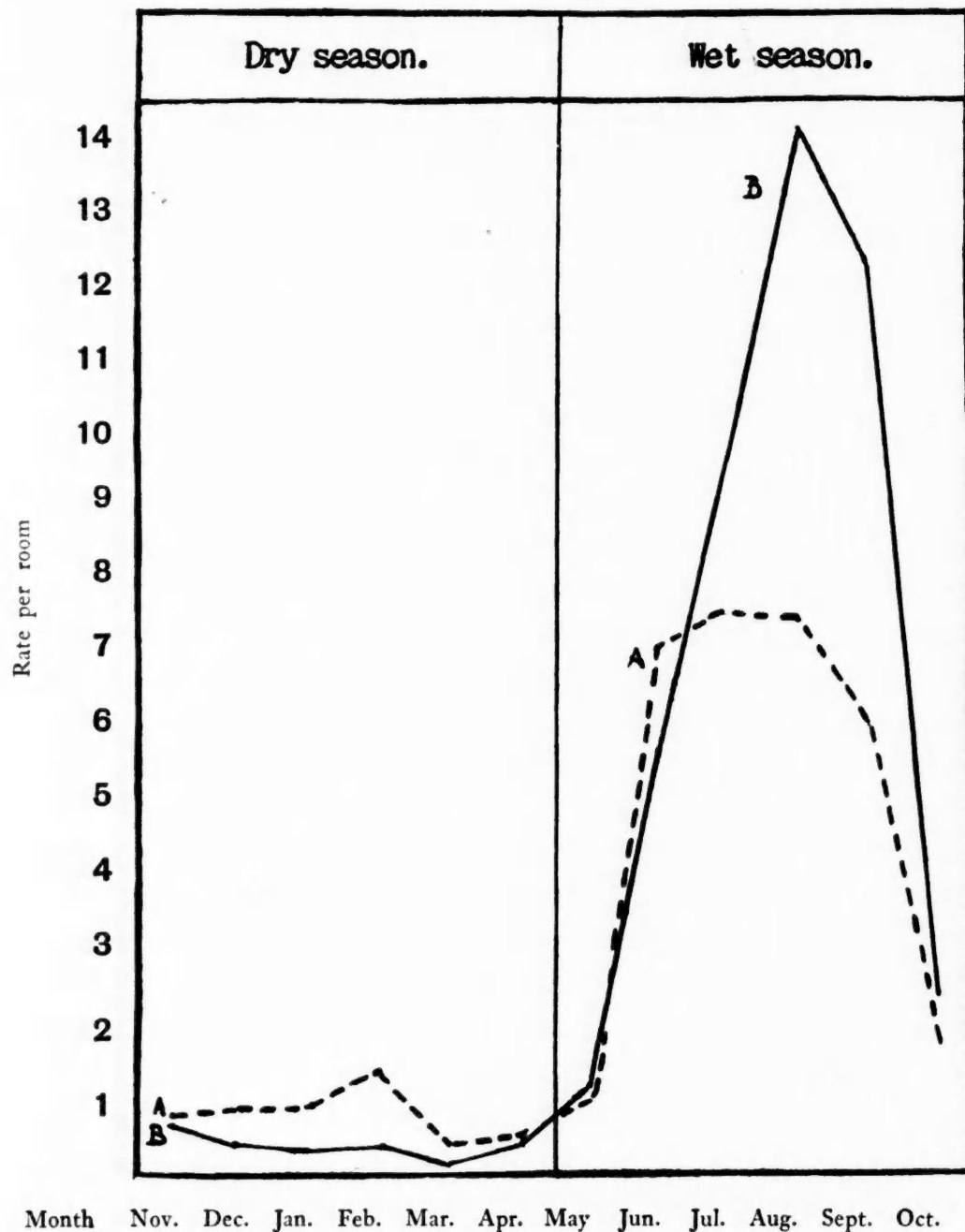
It appears justifiable to draw the following conclusions from Graph V. (1) Throughout the dry season, old anophelines predominate but young forms are constantly present, thereby indicating continuous breeding. (2) Throughout the rains, young anophelines predominate and there is a disproportionate rise in the number of old anophelines. (3) During the intermediate period, representing the close of the dry season and the commencement of the rains, old and young anophelines are about equally represented.

We have similarly analysed the Freetown figures regarding age incidence during the wet season, and have found that they follow much the same curve as that drawn for Kissy. It is unfortunate that the remarkable scarcity of anophelines during the dry season in Freetown prevented us from making observations over a twelve months period.

Up to this point our age classification of anophelines occurring in houses has been merely relative, and we have made no estimate of their actual ages. We had hoped, by keeping mosquitoes in captivity and examining their wing condition at intervals, to ascertain the actual ages represented by Perry's four grades. This, however, was not successful, probably owing to the impossibility of experimentally imitating natural conditions. We have, however, been

able to form some idea of the minimum age of the majority of the captured anophelines by means of Christopher's classification of the stages of development of the ovaries. By applying his standard, we found that, of 3,217 anophelines dissected, 1 per cent. were in Stage 1, 13 per cent. were in Stage 2, 5 per cent. were in Stage 3, 1 per cent. in Stage 4, 79 per cent. in Stage 5 (eggs ready for oviposition), and

GRAPH V



Showing the monthly rate per room of old (A) and young (B) anophelines at Kissy; based on the average monthly examination of forty rooms.

1 per cent. in Stage 5+. Since it takes at least six days to reach Stage 5, 80 per cent. must have lived for four or more days previous to capture. The actual age of the mosquitoes was probably far in excess of this minimum estimate, for the examination of 1,105 young anophelines (Wing Grade I) showed that no less than 74 per cent. had fully matured ovaries, and were therefore at least four days old at the time of capture; it follows therefore that the remaining 996 mosquitoes in Wing Grades II, III and IV must have existed longer than a minimum period of four days.

The proportion of anophelines found infected with malaria and filaria in various age groups. In the case of malaria, an anopheline once infected in the glands probably remains so throughout its life. Knowing that Grade I represents the youngest mosquitoes and Grade IV the oldest, it is to be expected that an increasing proportion of anophelines would be found infected in each successive grade. In the case of filaria, this does not necessarily follow, owing to the fact that the mosquito once infected does not normally retain the infection throughout the rest of its life. In Table XV we consider this question of the relation of age to infection.

TABLE XV

Showing the percentage of anophelines infected with malaria and filaria in different wing grades, and what proportion of these positives occurred in each wing grade; based on the dissection of 3,261 anophelines collected at Freetown and Kissy.

Wing Grade	Total anophelines	MALARIA			FILARIA		
		Number infected in glands	Percentage infected	Percentage of total infections	Number infected in head and/or proboscis	Percentage infected	Percentage of total infections
I	1,637	77	4.7	29.7	17	1.0	30.9
II	1,181	114	9.6	43.0	30	2.6	54.4
III	391	56	14.3	21.6	7	1.8	12.8
IV	52	13	25.0	5.0	1	1.9	1.8

Table XV shows a steadily increasing percentage of malaria infections in each successive wing grade, and is therefore a further argument in support of Perry's classification. It also shows that 5 per cent. of Grade I anophelines are infected in the glands, and that therefore this proportion of the youngest anophelines found in houses must have survived for about ten days.

It can be seen from Table XV that Grade II mosquitoes are by far the most important class as regards the transmission of both malaria and filariasis. In the case of malaria, Grade II has a far lower infection rate than Grade III or IV, but makes up in numbers what it lacks in intensity of infection. In the case of filaria, not only does Grade II contain the largest number of infections, but it is also the most intensely infected grade.

VIII. ASSOCIATION BETWEEN THE NUMBER OF NATIVE CHILDREN (GAMETOCYTE CARRIERS) AND THE INFECTION RATE AMONGST ANOPHELINE FOUND IN A HOUSE OR DISTRICT; TOGETHER WITH OBSERVATIONS ON THE DISPERSION OF ANOPHELINE FROM NATIVE HOUSES

It is of interest at this point to consider what proportion of the anophelines captured had fed once, or oftener, prior to capture. In order to gain information on this point we examined 800 anophelines immediately after capture, and found that 92 per cent. had taken blood not more than forty-eight hours previously. We have already shown that 79 per cent. of all anophelines captured had, as proved by the ovaries being in Stage 5, taken blood six or more days previous to dissection. If these percentages can be accepted as true for all the mosquitoes captured, it follows that 73 per cent. (i.e. 92 per cent. of 79 per cent.) of anophelines found in houses must have fed at least twice. Probably they had fed much oftener, for our observations with laboratory bred *A. costalis* and *A. funestus* have convinced us that a single blood meal is seldom sufficient to allow of complete maturation of the ovaries, a fact also observed by Davis (1928) in Argentine anophelines. This leads up to the very interesting question of whether these meals are partaken of in the same house, i.e. to what extent do anophelines haunt a particular house.

If the high proportion of anophelines which showed evidence of

more than one meal had obtained their previous meals in the same house, one would expect to find that their infection rate bore some relation to the infection rate of the inhabitants. In West Africa, the gametocyte carriers are almost entirely confined to children, a fact so frequently confirmed by writers on malaria that it requires no further comment. We therefore tried to find whether there was any correlation between the infection rate of the anophelines in the houses and the number of children who lived in them. The results are shown in Table XVI.

Table XVI shows clearly that there is no correlation between the number of children in a house and the infection rate amongst the anophelines therein captured. We have reproduced our figures in full, as they show that a low rate of infection may occur in houses with many children, and that a high rate of infection may occur amongst anophelines captured in houses only occupied by adults, thereby disproving a common belief that houses containing many gametocyte carriers necessarily show a high infection rate amongst the anophelines present. These results suggested to us that the infected anophelines had not necessarily acquired their infection in the house where they were caught. About twelve months ago, when carrying out some observations at an engineers' camp in the Port Loko district, which was occupied by about fifteen Europeans together with their native servants, we had interesting confirmation regarding this point. The camp was situated about two hundred yards from the edge of a swamp some one hundred yards wide, which we had proved to be the chief source of anophelines in the neighbourhood. On the other side of the swamp, and also at a distance of two hundred yards from it, was a small native village. In the European camp, we found the fairly high anopheline concentration (all *A. costalis*) of three per room (based on 241 anophelines caught). A similar anopheline concentration was found in the native village, which was to be expected, since the two places were equidistant from the swamp. The camp had been in existence for about six months, and as a result of residing there ourselves we are able to state, with absolute certainty, that no children were at any time present, while the number of native servants was comparatively small; also the blood of the Europeans had been recently examined and no gametocyte carriers detected. In view of these facts, we

TABLE XVI

Showing the number of adults and of children living in various houses in Kissy, together with the malaria infection rate amongst anophelines captured in these houses.

House no.	Number of visits	Total number of anophelines caught	Number of adults	Number of children	Percentage of anophelines infected with malaria
1	10	58	4	10	5'4
2	10	156	4	5	13'5
3	10	142	7	4	6'6
4	4	36	2	3	5'5
5	11	39	5	3	7'9
6	10	295	5	3	11'4
7	10	676	7	3	16'7
8	8	57	2	2	15'1
9	11	22	2	2	9'1
10	10	21	2	2	15'8
11	7	263	3	2	11'8
12	10	57	3	2	10'0
13	10	100	3	2	11'0
14	10	89	4	2	7'2
15	12	582	6	2	15'8
16	11	64	6	2	15'7
17	7	23	3	1	0
18	6	20	3	1	22'2
19	9	98	1	0	8'2
20	7	39	2	0	7'9
21	10	162	3	0	13'4
22	10	31	3	0	6'9
23	5	20	5	0	22'2

were surprised to find the exceedingly high sporozoite rate of 14 per cent. amongst the anophelines captured in the houses. This result caused us to suspect that the infected anophelines found in the European camp had not obtained their infection there, but must have obtained it from the only other available source, i.e. the native village five hundred yards away, on the other side of the swamp. This view was strengthened by the finding of a similar high rate of infection amongst the anophelines captured in this village.

Davis (1926), working in Brazil with *A. (Nyssorhynchus) argyritarsis*, states: 'It was found in a series of experiments that only 3.1 per cent. of the anopheline mosquitoes resting in certain houses on given days in the warm weather were present in the same houses on the days immediately following.' We have repeated at Kissy Davis's observation in Brazil, a number of experiments being carried out in the following manner. A native house having been selected, it was searched with meticulous care at seven o'clock in the morning, before the occupants had opened the doors and windows, and every anopheline caught was released into a gauze cage. It is impossible to guarantee that every mosquito in the house was removed, but the search was a very thorough one, and the number of anophelines which remained undetected must have been trivial. The captured anophelines were then sprayed with 1 per cent. aqueous eosin by means of an atomiser. A portion of the catch was retained as a control, and the remainder, when they were dry, were released in the rooms, approximately in the proportion in which they had been caught, all mosquitoes which appeared to suffer any ill-effects from the staining being discarded.

Our first experiments were conducted by re-examining the house, with the same meticulous care, after periods of twenty-four and forty-eight hours; the anophelines captured at the subsequent visits were killed and tested with alcohol for the presence of eosin. The control mosquitoes, retained from the first catch of each experiment, were similarly tested at the same time. All of thirty-two control anophelines from different experiments thus tested gave a sharp positive reaction, so that in Table XVII we do not think it necessary to record the fact that each experiment was controlled and the controls found positive. In this Table we only record the results obtained with *A. costalis* and *A. funestus*, and, since it was

found that the two species behaved in a similar manner, they are grouped together.

Table XVII proves that, whereas the number of anophelines in the two houses examined varied very little during the periods of examination, only a very small fraction (at no time more than 5 per cent.) of the original population was to be found, after respectively twenty-four and forty-eight hours. The twenty-four hour experiments prove that the vast majority of the anopheline population found in a house at seven o'clock in the morning had left it by seven o'clock the following morning. The forty-eight hour experiments prove that at any rate the vast majority of anophelines once having left a house do not return to it within a period of forty-eight hours.

TABLE XVII

Showing the proportion of the original anopheline population which was found present in a house after periods of twenty-four and forty-eight hours.

Experiment no.	House no.	FIRST EXAMINATION		Period between first and second examinations	SECOND EXAMINATION	
		Number of anophelines caught	Number of anophelines stained and replaced in house		Number of anophelines caught	Number of stained anophelines captured
1	1	79	74	24 hours	78	4*
2	2	168	152	24 hours	193	1
3	1	135	123	48 hours	113	2
4	1	120	105	48 hours	133	5
Total	...	502	454	24-48 hours ...	517	12

* Eight days later, when examining another house for a different purpose, some one hundred yards distant, another stained anopheline (which shows how well the stain is retained) from this particular batch was recovered.

As shown in Experiment 4, Table XVII, 105 stained anophelines were replaced in a house which had been completely cleared of mosquitoes; two days later it was again cleared, with the result that 5 stained anophelines were recaptured. There were therefore 100 stained anophelines still at liberty. In order to discover what proportion returned to the house during a period greater than forty-eight hours, we continued to clear the house at intervals, and

to examine the resultant catch for stained specimens. The result was as follows :—

3rd day catch		4th day catch		5th day catch		7th day catch		8th day catch	
Total	Number stained	Total	Number stained	Total	Number stained	Total	Number stained	Total	Number stained
133	2	89	1	45	2	61	0	64	0

It would appear, therefore, that of 105 anophelines in a house, 100 left it within a period of forty-eight hours, the remaining five either stayed in the house, or left and returned to it within a forty-eight hour period. Of the 100 stained anophelines still at liberty outside the house, only five are known to have returned to it during the next five days, whereas during the same period 392 unstained anophelines were taken in it. So far, therefore, as can be judged by a single experiment, the chances of an anopheline returning to the house where it has had its last meal are comparatively small. We have carefully considered certain points in Clayton Lane's (1931) interesting discussion of housing and malaria. He points out that (1) there is no clear evidence of a 'hospice return instinct.' Our experiments and observations in Sierra Leone strongly suggest that in West Africa no such instinct exists, and such returns as do occur are fortuitous. (2) He quotes various examples proving that the 'stay at hospice' habit varies in different countries and with different species of anophelines. Our observations in Sierra Leone appear to prove conclusively that *A. costalis* and *A. funestus* almost invariably leave the house within a short period of feeding. (3) Clayton Lane writes: 'Observations associating malaria in the inhabitants of a dwelling with malaria infection of the mosquitoes caught in the dwelling itself are, then, forthcoming from widely separated areas.' This may be true of other countries, but in Sierra Leone the absence of a 'hospice return instinct' and a 'stay at hospice' habit renders this association unlikely, while, in addition, our observations that a remarkably high infection rate may exist in a camp where there appeared to be no gametocyte carriers nearer than a village five hundred yards away, and the absence in Kissy of

any correlation between the infection rate of anophelines and the number of children (gametocyte carriers) in the houses, seem directly to disprove any such association. If we mass together the combined knowledge obtained from the dispersion experiments just described, the age of the mosquitoes, the condition of their ovaries, the presence or absence of evidence of a recent meal, and the relation of age to infection, it appears justifiable to sum up the behaviour of *A. costalis* and *A. funestus* in the houses of a native village in Sierra Leone in somewhat the following manner.

Every twenty-four hours there is, with the exception of a small residue not exceeding 4 per cent., a complete change over of the anopheline population in each house in the village. What happens to the anophelines that leave the house each night we cannot say with certainty, but it appears unlikely that the majority do so to oviposit. A minority probably seek some breeding place, but the majority enter some other house to obtain the further blood meals that are essential to complete the development of their ovaries. What happens to the remaining small number, about 4 per cent., which do not change over is a matter for conjecture, but it seems probable that, having entered the house with no desire for a meal, they wait twenty-four hours, and then, having obtained a meal, remain a further twenty-four hours to digest it before leaving the house.

IX. THE RELATIONSHIP OF THE TYPE OF NATIVE HOUSE AND ITS LIGHTING AND VENTILATION TO THE NUMBER OF ANOPHELINES FOUND IN IT

During our survey in Freetown, we kept records of the type of construction of the houses examined, and of certain points regarding their ventilation, etc. When we came to analyse our figures, and were able to compare together the effect on anopheline concentration of the types of construction of some six hundred houses, varying from the smallest mud hovel to two-storied concrete and stone houses, we found, as might be expected, that more anophelines had been taken in the poorer types of houses than in the more pretentious ; but these results were inconsistent and frequently contradictory. We finally reached the conclusion that what most affected anopheline concentration as estimated by early morning searches, was not so much the type of house as its (daylight) illumination and ventilation.

Below, in Table XVIII, we record the results obtained in some two hundred and fifty houses, where complete records had been made of their condition with regard to the degree of light and ventilation at the time of our examination. It should be noted that the houses examined for the purpose of this investigation were selected from streets where different types of houses occurred in close proximity to each other.

TABLE XVIII

Showing the percentage of native houses containing anophelines, and the anopheline rate per room, amongst 71 well-lighted houses as compared with 180 ill-lighted houses in Freetown.

	Number of houses examined	Number of anophelines caught	Number of houses containing anophelines	Percentage of houses containing anophelines	Anopheline rate per room
Well-lighted	71	68	23	32.4	0.96
Badly-lighted... ..	180	622	111	61.7	3.5

It is obvious from this Table that a far higher proportion of badly-lighted houses contained anophelines in the morning than did better lighted houses in the same vicinity, and that the anopheline concentration rate in the former was almost four times as great as in the latter.

These results would appear to be in accordance with the statements so often met with in the literature advocating light, airy houses in the tropics as important in the reduction of malaria infection. We do not, however, think that our results, although apparently corroborating these views, necessarily indicate that far fewer anophelines enter well-ventilated than badly-ventilated houses. What appears more likely is that anophelines enter such houses, but a higher proportion leave them at an early hour than do so in the case of dark, ill-ventilated houses. If this be the case, although the advantages of better native housing are obvious, it would appear unlikely that improvement in housing alone would result in any appreciable reduction in malaria incidence in Freetown, for our observations tend to show that the species in question, *A. costalis* and *A. funestus*, seldom remain in a house, whatever its condition, for more than twenty-four hours.

X. SUMMARY OF PART II

1. *A. costalis* and *A. funestus*, the predominant house-haunting anophelines in Freetown and the native village of Kissy, are both capable of experimental infection with *P. falciparum*, and have been proved by Hicks (1932) to allow the development of *W. bancrofti*, which is by far the most common species of filaria occurring in the district.

2. In Freetown and Kissy, both *A. costalis* and *A. funestus* show a high infection rate with malaria and filaria; another species *A. nili* also shows a high rate of infection with malaria, but its rarity in houses renders its importance negligible.

3. In all parts of West Africa from which figures are available, *A. costalis* and *A. funestus* show a higher rate of infection with malaria and filaria than any other species.

4. In Freetown and Kissy, the malaria infection rate amongst anophelines is similar in the wet and dry seasons. The filaria rate, on the contrary, is much higher in the wet than in the dry seasons. It is suggested that the relatively low humidity in the latter period is prejudicial to the development of the filaria in the anopheline host.

5. Evidence is adduced which supports Perry's (1912) statement that the relative ages of anophelines can be estimated by the stage of deterioration of their wing scales.

In Kissy and Freetown old anophelines predominate during the dry season, young anophelines during the rains.

A steadily increasing percentage of malaria infection occurs in each successive wing grade; this is not equally true of filaria infection. The great majority of anophelines infected with malaria and filaria are in Grade II; this, therefore, although not the most highly infected grade, is the grade most responsible for the transmission of these two diseases.

6. No association is demonstrated between the number of native children (gametocyte carriers) and the infection rate amongst anophelines found in a house or district.

7. The great majority of *A. costalis* and *A. funestus* leave a native house within twenty-four hours of having fed. There is no evidence of any return habit. On the basis of our known facts regarding the age, infection rate, dispersion and feeding habits, etc.,

of the species in question, it is surmised that an almost complete change-over of the anopheline population of each house occurs every twenty-four hours.

8. A comparison of well-lighted and badly-lighted houses in Freetown shows that a higher proportion of the latter contain anophelines, and that in such houses the anopheline concentration is almost four times as great as in well-lighted houses in the same vicinity. It is, however, suggested that this does not necessarily indicate that fewer anophelines enter well-ventilated houses, but rather that they leave them at an earlier hour.

PART III

THE HISTORY OF ANTI-MOSQUITO MEASURES IN
FREETOWN FROM THEIR INCEPTION IN 1899 UP
TO THE PRESENT TIME, TOGETHER WITH
CERTAIN OBSERVATIONS ON THEIR RESULTS

I. INTRODUCTION

Anti-mosquito work in Freetown may be said to have started in 1899, when Sir Ronald Ross visited the Colony of Sierra Leone in order to continue the work on malaria which he had begun in India. Since then sanitary measures have been carried out with a degree of intensity proportionate to the funds available, but, unfortunately, until 1930 such funds were never sufficient to allow of any large undertaking. In 1930, however, a very comprehensive scheme of anti-mosquito drainage was commenced. This was intended to supply permanent street drains of the most modern type over the whole of Freetown, and to canalise the streams traversing the town. The work was to be carried out in three sections, the first to be completed in five years at an estimated cost of £60,000; it was decided that the first area to be dealt with should be in the western portion of the town. A district was selected covering about three-quarters of a mile square; this area is shown in the attached large scale map (Map II), on which we have recorded certain points of interest regarding past and present conditions. The district under consideration has always been recognised as one of the most malarious in Freetown, and corresponds closely to the hyperendemic area described by Macdonald (1926). The south-western part of this area, as shown in Map II, is traversed by Sander's Brook, and the land here is more level than elsewhere, the street drains more primitive, and the surface of the streets more uneven. This portion of the district was even more in need of anti-mosquito drainage than the rest of the area, where the ground was more sloping and a larger number of cement drains had been constructed. These differences in the two areas are reflected in the number of mosquitoes caught in them, as may be seen by consulting the figures shown on the map.

At the suggestion of the Medical Department, it was decided to carry out an investigation to estimate the effects which this extensive drainage scheme might have in reducing anophelism, and therefore malaria, in Freetown. We proposed to do this in two ways: (1) by investigating the number of mosquitoes occurring in the houses before and after the establishment of improved drainage in the area; (2) by estimating the intensity of malaria (as shown by the parasite rate and spleen examination) amongst the children before the improved drainage had been introduced, and again some five or more years afterwards. This latter part of the work would have been greatly facilitated by the fact that we already had a report by Macdonald (1926), which represented the results of his very careful examination of the blood and spleen measurements of over 1,000 native children, his investigation having been made in the exact area which it was proposed first to canalise.

The results which follow are based on the first two years' work; but unfortunately the anti-malaria scheme was suspended in 1931 owing to a shortage of funds which had resulted from the general economic crisis. On account of this unforeseen curtailment of the scheme, our figures are incomplete, but we believe that they are sufficient to assess the value of that part of the 1930 scheme so far completed, while the survey made prior to its commencement enables us to estimate what improvements have taken place since the days of early observers.

We have already mentioned that our mosquito survey of Freetown was later extended to include the neighbouring native village of Kissy. Our reasons for this extension were two-fold: in the first place the absence of anophelines from houses in Freetown during the dry season rendered it necessary that we should obtain our material for certain investigations from some other source; secondly, we came to the conclusion that the surprising absence of mosquitoes in Freetown was due to the anti-malaria measures of recent years, and, after careful study of Kissy and of the reports of the early writers, we decided that the Kissy of to-day is in very much the same condition as the Freetown of some thirty years ago, when Ross, Stephens and others first endeavoured to remove from it the opprobrious epithet of the 'White Man's Grave.'

II. CONDITIONS IN FREETOWN IN 1899-1904

The following description of Freetown in 1899-1904 is compiled from the reports of Ross, Annett and Austen (1900), Stephens and Christophers (1900), Ross (1901 and 1928), Logan Taylor (1902), and Boyce, Evans and Clarke (1905).

The central part of the town, which lay on the lower slopes of Tower Hill and in which most of the Europeans resided, was built upon 'clayey earth,' through which the lava rock (laterite) only occasionally protruded. The roads were even and for the most part had roughly excavated drains at their sides; few anopheline breeding-places were found in this part of the town.

The east and west portions of the town were flat, while the streets were merely open spaces between the houses formed by the natural laterite rock, unpaved and generally undrained. The weathering of the rock allowed the formation of many cavities, and the few drains were ditch-like and flat-bottomed. Two main streams flowed through the town, Nicol's Brook on the east and Sander's Brook on the west, the beds of which were formed of bare irregular rock. There were also a number of runnels of water from springs, or waste water descending from the Barracks on Tower Hill.

The population was 30,033 in 1891, and had presumably increased at the time that these reports were written. The native inhabitants mostly lived in houses which were thatched, built of wood on a mud or rough stone foundation, and separated from each other by fences enclosing compounds. Only a small proportion of their refuse was removed by the Municipal Health Department, and most compounds, not having been cleared for twenty or thirty years, contained a striking collection of old bottles, tins, broken earthenware, pots and other refuse. Almost every compound had a cesspit, of which there were about 3,000 in the town, and close to which a well was often situated. There was also a pipe-borne supply of pure water from the hills, but only about seventy taps had been fitted to this system.

Under these conditions anophelines bred in numbers both in the wet and dry seasons. In the dry season, they bred in the pools formed in the rocky beds of the streams and in springs. The commencement of the rains, however, scoured out these pools and

washed away the larvae, but on the other hand they filled up cavities in the rock of the streets and compounds, and created innumerable puddles. In these puddles, which by the nature of the ground were not liable to scouring, anopheline larvae flourished. This difference between the conditions in the wet and dry seasons is well shown by the maps published by Ross, Annett and Austen (1900), and by Stephens and Christophers (1900). The latter worked in a selected area in the dry season, and their map shows that in this test district adult anophelines were only found in houses close to Sander's Brook. Houses remote from the stream very rarely harboured them, and in their vicinity larvae could only be obtained when pools were artificially made. The former authors, however, working in the wet season, described many breeding pools scattered throughout this same area. Boyce, Evans and Clarke (1905) stated that anophelines bred also in almost all the open wells examined.

Culicine and *Stegomyia* larvae were present during the wet season in the water held in discarded bottles, tins, water casks and other receptacles, and during the dry season in wells, puddles and pools in the streams. Culicines were also found breeding in large numbers in the cesspits.

As a result of their experiences in Freetown, Ross, Annett and Austen (1900) and Stephens and Christophers (1900) suggested, amongst other reforms, that a vigorous and sustained attack should be made on mosquito larvae. The destruction of breeding places by drainage, filling up pools, levelling street surfaces, and removal of rubbish was recommended as a fundamental measure, to be supported by subsidiary proceedings such as the brushing out of small puddles and the application of kerosene or tar as larvicides.

When Ross found that his recommendations had been ignored and that no official anti-mosquito work was to be undertaken, he returned in 1901 with Dr. Logan Taylor, determined 'to hire labourers there, to buy pickaxes and shovels, to show them how to reduce mosquitoes . . . ' (Ross, 1928). The money necessary to carry out this work was received from a private source. A party of thirty to forty native labourers was collected, and under the direction of Dr. Logan Taylor some of the more important streets were drained by cuttings in the rock or earth. A gang of men, the 'Culex gang,' visited the houses with a cart, and cleared the

compounds of their accumulation of refuse, while an ' *Anopheles* gang ' was organised which dealt with every pool twice a week. The smaller pools were swept out with a brush ; in the case of the larger, some were treated with kerosene or creosote, some were filled in, and some evacuated by making an outlet channel through the rock or earth which contained them. This work began early in July, 1901, and in a letter quoted by Ross (1901), Daniels, who had inspected the work, wrote : ' In my opinion, already your efforts have been crowned with a large degree of success, as there has been a noteworthy diminution in the number of the first two genera (*Anopheles* and *Stegomyia*) found in the houses. The number of breeding grounds has been enormously diminished.' He stated, however, that a great deal remained to be done ; many breeding places were still present, the rock drains were too narrow, and the earth drains liable to fall in. For the treatment of the streams in the dry weather, he suggested two schemes, the formation of a central channel in the bed of the stream, or a dam to collect a sufficient head of water to flush out the stream intermittently.

Boyce, Evans and Clarke (1905) found that many drains had been completed, but that a large number were unsatisfactory, having insufficient fall or being choked with refuse so that pools of water collected, while no attempt had been made to deal with the streams. The disposal of refuse had been facilitated by the provision of dust bins in various parts of the town, and measures had been taken to prevent householders from accumulating rubbish in their yards. They recommended the following anti-mosquito measures :—

' (1) The reconstruction of the bed of the streams. (2) The abolition of wells. (3) The closure of the cess-pits. (4) The reconstruction of the roads, the repairing and making of new street drains, and the draining of the compounds.'

III. CONDITIONS IN FREETOWN IN LATER YEARS

The progress of the work can henceforth be studied in the annual reports of the Medical and Sanitary Department of the Colony, and certain reports issued by the staff of the Sir Alfred Jones Research Laboratory. The measures adopted during recent years have included the following :—

(1) Oiling. (2) Drainage. (3) Filling puddles. (4) Removal of

refuse, and daily sanitary inspections of compounds. (5) Prosecution of householders for allowing larvae to breed in domestic utensils. (6) Closure of wells. (7) Clearing high grass and bush. (8) Building of public latrines to take the place of cesspits.

(1) The oiling of pools, cesspits, etc., is carried out systematically, using a mixture of kerosene and disinfectant. (2) The provision of permanent drains has always been retarded by lack of funds. In the earlier years, drains had been cut in earth or in solid rock, but these were not found to be satisfactory. Earth drains needed constant attention to keep them open, while rock drains became eroded and allowed the formation of puddles. Until recently only a few concrete drains were in existence. (3) Puddles and other hollows are filled in by sanitary gangs, and a supply of rubble, small stones and sand from the Government quarry is placed at the disposal of the inhabitants, who freely make use of it for this purpose. Cavities in trees are drained or filled with a mixture of tar and cement. (4) and (5) Sanitary dust bins are provided and emptied by the Health Department. It has been made an offence to allow in compounds the accumulation of rubbish or the breeding of mosquitoes in water contained in domestic utensils, tins or bottles, etc., and fines are constantly being imposed for breaches of this ordinance. (6) In 1913, 7.6 per cent. of the 418 wells examined contained anopheline larvae. As the pipe supply of water was extended, it was possible to close nearly all these wells. In 1918, 'the distance from the nearest standpipe within which it was obligatory on owners in Freetown to have their wells closed was increased from 130 to 500 yards,' and 145 wells were closed. There was some concealment of wells by owners, so that it was difficult for the Health Department to ascertain the exact number in existence. In 1920, the annual report states that 176 wells were closed, leaving about fifty-five still open; in 1921, forty-three were closed, leaving twelve open. At the present time only three wells are known to be open. (7) Government lands, where high grass and bush conceal breeding-places, are cleared by sanitary gangs, householders being made responsible for clearing their private property. In 1930, over 10,000 notices were served upon the occupiers of compounds for not clearing grass and weeds. (8) About 4,000 cesspits are known to be still in use, and remain as potential breeding-places. They are, however, oiled

regularly, and public latrines are being built, as funds allow, to take their place.

The deterrent effect of this work on mosquito breeding is shown by the larval surveys of Blacklock (1921), Blacklock and Evans (1926), the larval indices in the various annual reports of the Medical and Sanitary Department, and by our mosquito survey in 1930 and 1931.

Blacklock (1921), at the end of the dry season, found anophelines breeding chiefly in the streams, and especially in two parts of their courses: 'The first is the edge of the winding and eroded bed of the stream just before its entrance to the sea, the second is the shallow water, well protected by vegetation and extending over a large surface, which is found at the places of origin of these streams.' In the town proper the breeding places were scanty. Here 'the streams are passing through a rocky formation, with the result that there is slower erosion; this and the great amount of canalisation of tributaries which has been carried out in recent years permit of more complete washing out of the stream bed in its passage through the town.'

Blacklock and Evans (1926), in the rainy season, made an extensive survey of breeding places. They found larvae especially abundant at the junctions of lateral drains with the main stream of Sander's Brook, and in addition in pools in laterite street drains and in shallow pits. They considered that 'it is clear that there has been an enormous reduction of adults and breeding places' since the time of the earlier observers. Below, in Table XIX, we give figures collected from the various annual medical reports, showing the reduction which has taken place during recent years in the number of breeding places found in compounds.

Table XIX shows that, although many discrepancies occurred, there has been a steady diminution in the proportion of compounds which harboured larvae.

IV. CONDITIONS IN FREETOWN DURING THE SURVEY OF 1930 AND 1931

The results of dissection of anophelines during the survey have already been fully discussed, and we do not propose to mention them further in this article, except to point out that there was no evidence of any increase in the proportion of anophelines found

infected in 1930 as compared with the findings of the observers as far back as 1900. These early observations give few actual figures of mosquito concentration, and in consequence we can only compare our observations with theirs in general terms. In order more accurately to estimate the results of sanitation in Freetown, we

TABLE XIX

Showing the percentage of compounds, visited for the special purpose of taking the index,* which were found to contain mosquito larvae. (Compiled from the annual reports of the Medical and Sanitary Department.)

Year	Number of compounds visited for the purpose of taking each index	PERCENTAGE OF COMPOUNDS WHICH CONTAINED LARVAE (Less than 0.1 per cent. marked as nought)											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1911	20	14.8	16.4	...	6.8
1912	200-250	15.5	...	6.4	...	5.2
1913	250	...	2.8	5.6	4	3.2
1914	250-350	0.3	11.6	25.2	3.2
1920	350	0	8.9	2.3	2.9
1921	350	1.2	4	2.2
1922	300	0.6	2.3	0.6	0.4
1923	350	1.4	3.7	2.6	1.1
1924	1.4	1.4
1925	350	1.6	1.1	0.9	1.4
1926	350	0.6	1.3	2.3	2.3
1927	350	0	0	0	0
1928	350	0	0	0	1.3
1929	350	0.6	1.4	0.3	0.6
1930	350	0.3	2.9	0.9	0.3

*Apart from the 600 compounds which are inspected daily as a routine

thought it desirable to institute a similar survey at Kissy, a large village in the neighbourhood, which by its primitive sanitation resembled closely the Freetown of 1900, thus allowing the same workers with the same technique to examine in the same year two

distinct sanitary epochs. A certain number of houses were chosen and visited regularly, the search being carried out in the same way as in the Freetown survey.

The houses in Kissy were in most instances rough wooden shacks, with a roof of thatch or, less often, galvanised iron; all the streets except two consisted of bare rock, containing numerous hollows; an uncanalised stream ran through the area and passed close to a swamp in the level ground below the town. Mosquito larvae were plentiful during the rains; anophelines bred in pools in the roads and compounds, in the swamp, in water trickling through the cultivated land, and in the stream where the flow of water was not too strong; everywhere culicine mosquitoes bred in puddles in roads and compounds.

How closely this description of the sanitary condition of Kissy at present corresponds to that of Freetown some thirty years ago can be seen by comparing it with the account, already quoted, given by the early writers.

In the following brief summary of our investigation, in so far as it bears on the results of sanitation, we contrast the number of adult anophelines found in Freetown and Kissy in 1930 and 1931 and compare them with the observations of Ross, Stephens and Christophers, and others. We have omitted all reference to culicines, as the early observers made little or no mention of them and our own results in Kissy and Freetown have been fully discussed in Part I.

In Kissy, during the dry season, the anopheline rate per room was 1.08, and the maximum taken in a house averaged 10 per room. In Freetown, during the same season, anophelines were extremely scarce and the rate per room never rose above 0.1, while the maximum taken in a house never averaged more than 2 per room. These Freetown figures may be compared with the results obtained in the same area by Stephens and Christophers (1900), also working in the dry season. They wrote: 'In the neighbourhood, however, of the brook and drain, anopheles were to be detected in most of the houses. But even here, in well-built, clean houses, it was difficult to obtain them in the early morning, such anopheles as entered at night appearing not to remain. In small dirty and dark houses, however, a variable number were always to be obtained . . . In such places female anopheles were found in large numbers, while

in houses immediately adjoining they were difficult to obtain . . . In native dwellings—and in these overcrowding is often extreme—anopheles are frequently present in enormous numbers.' In one of the sheds they found seventy anophelines. They attach a plan to their report which shows the exact site of the houses in which anophelines occurred 'in enormous numbers.' By means of their map we have been able to visit this area and to examine what were in all probability the original houses, without finding a single anopheline in the dry season (the time of their survey) and only a few in the wet season. This observation is demonstrated on the attached map.

During the rains there was a very marked rise of the anopheline incidence in both Freetown and Kissy, but whereas in Freetown anophelines only averaged 0.4 per room, and never exceeded a maximum average of 16 per room, in Kissy the rate per room was 10.5 and the maximum average was 48 per room. In a few houses in Freetown the number of anophelines taken was considerably above the average for other houses in the area, and in eight of the 603 houses examined, twenty or more female anophelines were captured, the actual numbers being as follows, with the number of rooms examined given in the bracket: 48 (3), 41 (4), 28 (4), 26 (4), 24 (6), 24 (4), 22 (4), 20 (2). But these never approached the maximum figures for Kissy, which were: 194 (4), 168 (4), 135 (3), 133 (3), 133 (3), 123 (3), 120 (3), 113 (3). It is unfortunate that the early writers gave no indication of the number of anophelines to be found in Freetown during the rains, but, judging from their remarks, they were probably very numerous. It is significant that Ross, Annett and Austen (1900), working in both Freetown and Kissy, make no comment on any difference between the two places in respect of the number of anophelines.

The above figures appear to prove conclusively that a remarkable diminution in the number of mosquitoes in Freetown has taken place during the past thirty years. Undoubtedly the change has been a gradual one, and it is difficult to assess the part played by the sanitary measures employed. The most easily estimated is certainly drainage. Thus the improvement noted by us in the intensely infested area recorded by Stephens and Christophers must undoubtedly have been due to drainage, as by their own statement Sander's Brook, at that time uncanalised, was the only possible source of the adult anophelines found.

It was of course hoped that, as the result of a very comprehensive survey of the numbers of anophelines in houses before and after the completion of the anti-malaria drainage scheme, we should have figures of such magnitude that they would furnish a really reliable criterion of the extent of the improvement, which was expected to follow a drainage scheme so extensive and permanent that it would render unnecessary the ever-recurring expense of oiling and temporary canalisation. Most unfortunately, as we have mentioned, the scheme broke down through lack of funds, and the comprehensive figures hoped for were not obtained. Fortunately, however, we have obtained adequate anopheline concentration figures for some of the streets in which no modern drainage had been attempted before our first visit in 1930, but in which such drainage had been completed before the time of our second visit in 1931. In contrast to these, we have control observations during two years regarding streets in the same area and of similar type, in which no drainage operations had been attempted. Before considering these figures, it is necessary to draw attention to the fact that a marked rise in the anopheline concentration rate was noted throughout the whole area under survey in the year 1931 as compared with 1930. In 1930, the anopheline rate per room was 0.33 based on 502 anophelines captured, while in 1931 it was 0.47 based on 712 anophelines captured. This general rise in the anopheline rate per room throughout the area surveyed does not prevent our calculating the effects of drainage, for our figures enable us to compare together the ratios of the anopheline rates per room in 1930 and 1931, (1) in streets in which no drainage had been carried out, and (2) in streets in which drainage had been completed. This comparison is indicative of the effect of drainage, and is unaffected by the general rise referred to. The anopheline concentration in the drained and undrained streets is shown in Table XX.

Table XX shows that, in streets in which the drainage condition was unchanged in 1930 and 1931, the anopheline rate per room was two and three-quarter times as great in 1931 as in 1930; whereas those streets in the same area, in which the drains were constructed in 1930 following our first examination, showed in 1931 not an increase, but on the contrary a fall to almost half of the previous year's concentration. It would appear that this improvement is directly due to the drainage effected during the twelve months

separating the times of the two surveys, but due allowance must be made for factors affecting the accuracy of the observations, such as the small number of streets and the fact that mosquito breeding is liable to be unevenly increased during constructional work.

TABLE XX

Showing the anopheline rate per room in (A) streets in which no drainage operations were carried out in 1930 or 1931, (B) streets in which drains were constructed in 1930.

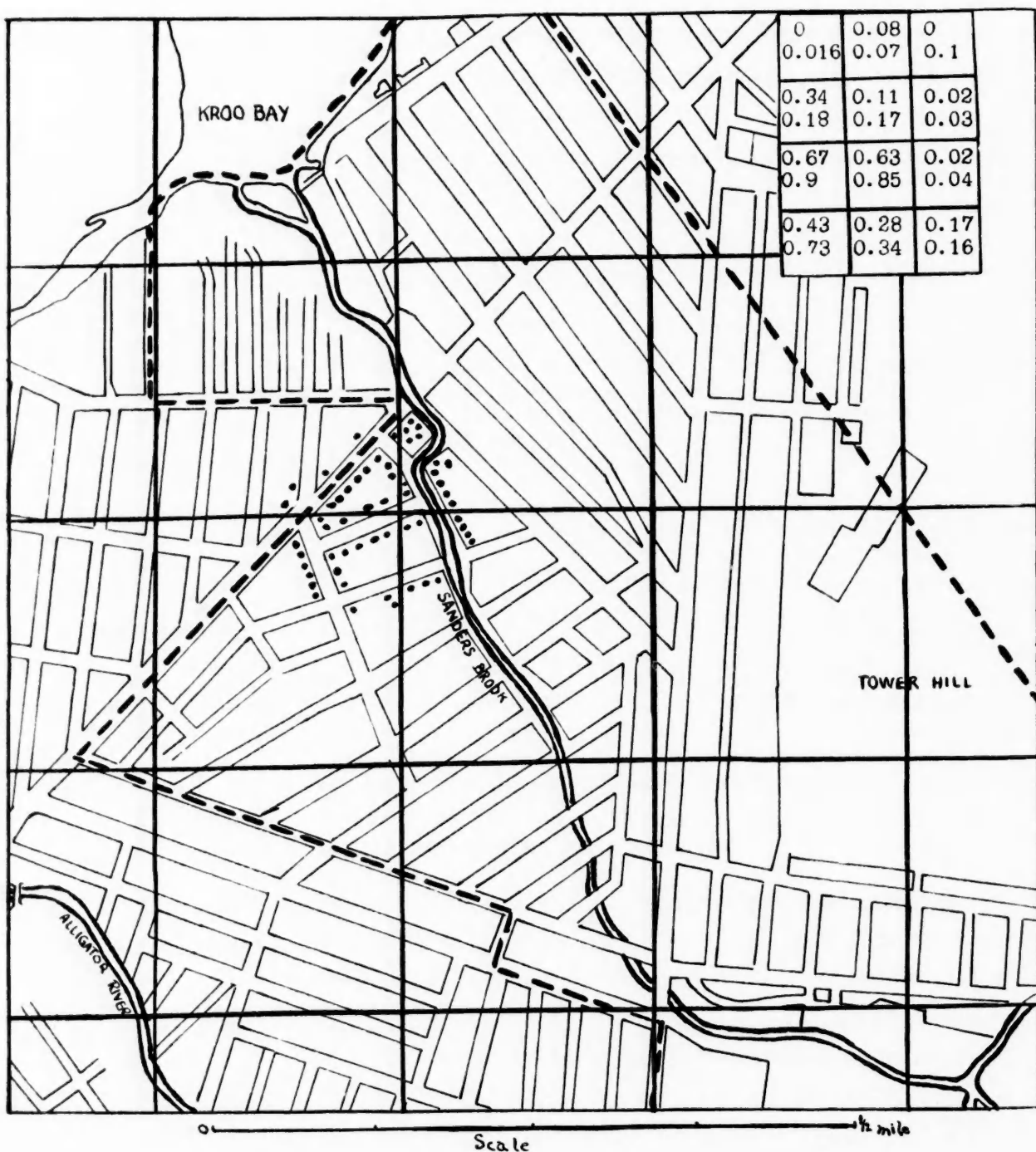
Column 1	1930				1931			Ratio 1931 to 1930, i.e. column 8 column 5
	2 Number of streets examined	3 Number of rooms searched	4 Total anophelines caught	5 Anopheline rate per room	6 Number of streets examined	7 Total anophelines caught	8 Anopheline rate per room	
A. Streets in which no drainage operations were carried out in 1930 and 1931 ...	13	535	122	0.23	570	360	0.63	2.74
B. Streets in which drains were constructed in 1930 ...	6	182	159	0.87	186	89	0.48	0.55

V. SUMMARY OF PART III

1. An account is given of the condition of Freetown and the prevalence of mosquitoes in 1899-1904, with the methods adopted to combat their breeding. The account is brought up to the present time, based on the reports of the Medical and Sanitary Department and those of other observers.

2. A mosquito survey of the western area of Freetown in 1930 and 1931 shows a very great reduction in the number of anophelines, due to the routine anti-mosquito work carried out. In the neighbouring village of Kissy, where permanent drainage and similar methods have not been applied, anophelines are still very common.

3. Such portions of the 1930 anti-malaria drainage scheme as were completed prior to its abandonment are shown to have proved successful, in that there was a reduction in the number of anophelines captured in the streets drained.



MAP II. Diagram of a portion of Freetown showing, within the interrupted line, the area selected for canalisation, and surveyed in 1930 and 1931. The squares inset in the right hand upper corner correspond to those on the diagram, and show for the areas thus indicated the anopheline rates per room for 1930 (the upper figure) and for 1931 (the lower figure). The black dots along the streets in the centre of the area represent those houses in which anophelines were found by Stephens and Christophers in great numbers during the dry season of 1899-1900, but in which in 1930-1931 none were found in the dry season and very few during the rains.

SUMMARY AND DISCUSSION

Freetown is usually regarded as a highly malarious town and one in which mosquitoes are very prevalent. There are adequate figures showing that a high proportion of the native children are infected with malaria, but knowledge regarding the number of adult mosquitoes was, up to the time of our survey, based on casual observations and the results of larval surveys, a method liable to many errors. When we began our survey, in view of the high malaria rate amongst the children, we expected to find the anopheline concentration in native houses in Freetown to be extremely high, and we were surprised to discover that, on the contrary, it was extremely low, that is to say, low in comparison with the neighbouring village of Kissy and with Lagos and Ibadan, the only two large West African towns from which figures are available; this difference appears to be reflected in the higher malaria infection rate amongst children in Lagos as compared with Freetown. On the other hand, the malaria infection rate amongst the anophelines captured in houses in Freetown was comparatively high and similar to that noted in Kissy and in the Nigerian towns referred to. This comparatively high infection rate in Sierra Leone did not appear subject to much seasonal variation. The filaria rate amongst anophelines in Freetown was also high, but not so high as that noted in Kissy, where amongst the large number of anophelines captured there was observed a very marked rise in the infection rate during the wet season.

Our investigations have shown that in Freetown and Kissy the only species of anophelines of importance in the transmission of malaria and of filariasis are *A. costalis* and *A. funestus*. Further, an examination of the literature would appear to show conclusively that throughout the whole of West Africa these two species are, for all practical purposes, solely responsible for the transmission of malaria; in all probability, the important part which we have shown they take in the transmission of filariasis in Sierra Leone is also taken by these two species in the other West African Colonies, but here the issue is complicated by the lack of accurate information regarding the numbers and the infection rate of certain potential

culicine vectors which are not numerous in Sierra Leone. A limited number of experiments, regarding the species and numbers of mosquitoes biting out of doors, suggest that transmission may be carried on in Freetown and Kissy to a small degree by certain species which seldom, if ever, appear in houses.

Having shown the supreme importance of *A. costalis* and *A. funestus* in the transmission of malaria and filariasis, we next investigated certain points in their bionomics with relation to the spread of these diseases. It was found that individuals of these two species seldom remained in a house for more than twenty-four hours and that their return to the same house was a matter of chance, an observation borne out by the lack of association between the number of children (gametocyte carriers) in a house or district and the infection rate amongst the anophelines captured. It would appear to follow, therefore, that the so-called 'malaria house' in Sierra Leone owes its reputation to a high anopheline concentration as compared with surrounding houses, rather than to a high incidence of infection amongst the anophelines in it.

In understanding some of the problems in the life-history of anophelines and their bearing on the transmission of malaria, it would be of great value to be able accurately to estimate the length of life of the adults in nature; at present no method of doing this is known, but it would appear possible to estimate their relative ages, using the method outlined by Perry. By applying his criteria to the anophelines captured in Freetown and Kissy, results were obtained which were found to be in accordance with known facts regarding breeding, feeding, habits, etc., and suggested that during the dry season little breeding went on, but that it was sufficient to maintain a constant though small supply of young mosquitoes in the houses. With the advent of the rains, both young and old forms were greatly increased in numbers, but the emergence of fresh anophelines was so rapidly augmented that finally they completely outnumbered the previously predominate old forms. When this method of age classification was applied to the malaria and filaria infection rates, it was found that the greatest number of these infections was associated with comparatively young mosquitoes (Grade II), which is therefore the age class chiefly responsible for the transmission of these two diseases. Their importance, however,

in this respect, depends only on their numerical superiority, for, as might be expected, it is the oldest mosquitoes (Grade IV) which show the highest proportion of infections with malaria.

A great reduction has taken place in Freetown during the past thirty years in the number of anophelines and culicines, but the entire absence of figures, as opposed to mere statements, in the work of early observers prevents our estimating it accurately. The change has been a gradual one, and undoubtedly the steady improvement noted has been due to anti-larval measures, especially drainage. Observations on the relation of housing to mosquito concentration suggest that improved housing has little direct bearing on the number of anophelines found in houses—a matter which appears to depend directly on the amount of illumination in the house, and therefore primarily on the habits of the people.

Yellow fever has not been reported from Sierra Leone for more than ten years, although other territories adjacent to it have not been so fortunate. Our mosquito survey enabled us to estimate the concentration of the Culicini occurring in houses and biting out of doors, and the numbers amongst them of species known to be potential yellow fever vectors. It was found that the number of potential vectors was represented by the almost negligible figures of 1.3 per 1,000 rooms, a surprisingly low figure when compared with that which we have quoted for Lagos (Nigeria) of 380 potential vectors per 1,000 rooms. It was this remarkable result which suggested to us the advisability of undertaking the outdoor biting experiments in which we found that, although not numerous, the potential vectors found biting out of doors markedly outnumbered those found in houses. It would be extremely unwise on such evidence to ascribe the freedom of Freetown from yellow fever in the past to the scarcity of vectors in that city; but our results at least suggest the importance of estimating the number of adult mosquitoes found in houses or biting out of doors, instead of placing reliance on larval surveys.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS *MUSCA* BASED ON A COMPARATIVE STUDY OF THE MALE TERMINALIA

I. THE NATURAL GROUPING OF THE SPECIES AND THEIR RELATIONSHIP TO EACH OTHER

BY

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INTRODUCTION

Musca is, next to *Glossina*, the most important Muscine genus, and that for the following reasons :—(1) It contains the notorious pest, *Musca domestica*, and its tropical and subtropical allies, *Musca vicina*, *M. nebulo*, *M. sorbens* and *M. vetustissima*, all of which are widely distributed, house-frequenting flies, and are certain transmitters of pathogenic organisms to human food and the human body. *Musca domestica* is a true biological carrier of *Habronema* to Equines in different parts of the world. (2) The genus contains a number of haematophagous species such as *Musca autumnalis*, *M. bezzii*, *M. lusoria* and many others, which, though being unable to scratch through into the skin of animals and draw blood, follow the true blood-sucking flies, and cause them to withdraw the proboscis in order that they may then suck up any blood or serum which commonly exudes from the bite. These interesting flies are only found out of doors, and may be seen flitting about from one spot to another on the bodies of animals searching for all possible sources of blood, serum and other exudations. They are obviously, in virtue of this scavenging habit, potential transmitters of pathogenic trypanosomes, and possibly other parasites, to animals; their potentialities in this direction have yet to be explored. Further,

it is only necessary to watch one of these species swarming around the eyes and nostrils, and on cuts and abrasions, etc., of cattle to appreciate how intensely they worry them, and in the case of milch cows materially reduce the output of milk. (3) The genus contains five (the number known at present) true blood-sucking species which are able to scratch the skin and draw blood on which they greedily feed, and they are, therefore, not dependent on other flies for their food. These species, too, are potential transmitters of pathogenic organisms both mechanically and biologically, but as yet nothing is known of their capabilities in this direction. *Musca crassirostris* is a probable transmitter of *Habronema* in India, for I have found these nematodes developing in it. Both these haematophagous and blood-sucking species have an added interest to the morphologist for they exhibit the evolution of the scratching proboscis in the Muscinae, an apparatus which reaches its highest development in *Glossina*. The beginnings of this scratching and tearing apparatus, for that is its true function, can be seen on the labella of some of the haematophagous species such as *M. mesopotamiensis*, and its further development can be followed in the labella of *M. conducens* on to the more elaborate scratching armature of the blood-sucking forms such as *M. senior-whitei*, *M. fletcheri*, *M. planiceps*, *M. inferior* and *M. crassirostris*. Although there is a considerable gap at the end of this series its further development can be traced through *Stomoxys* to *Glossina*. And it is necessary to point out here that were it not for this remarkable series of stepping stones in the evolution of this very efficient apparatus, it would be quite impossible to understand the highly developed and complex scratching, tearing and piercing armature on the labella of *Glossina* were it studied by itself. This type of tearing apparatus is also well developed on the labella of many strictly predaceous higher Diptera such as *Scatophaga*, *Lispa*, *Coenosia*, *Bengalia*, etc. (4) Lastly, the genus is of the first importance to the systematist, for, in the simpler species, the terminalia (external genital armature) exhibit an early phase in the evolution of these structures of the greatest possible use in interpreting the corresponding structures in the more complex Muscinae, and in the Anthomyinae, Calliphorinae, Sarcophaginae and Tachininae. The true significance of these terminalic characters in this direction will be emphasised in this and in subsequent papers

of this series. For all these reasons it is important that all the species of this genus should be known, and their true relationships to each other and allied Muscinae clearly understood. Research into the parasite-transmitting potentialities of these flies will then be carried out more intelligently and with greater precision.

My interest in the species dates back to 1905 when I had the opportunity of studying the habits of three common species in South India, viz., *M. conducens*, *M. pattoni* and *M. crassirostris*. Although at that early date I did not know the true systematic position of these flies, and indeed did not even know their names, I had many opportunities of studying them in the field, observing their feeding and breeding habits, collecting and studying their early stages, and, most important of all, becoming so familiar with them that identification was always certain. In 1913, in a paper with the late Major Cragg, I.M.S., I described the habits and early stages of some haematophagous species, and drew attention to their peculiar scavenging habits, and their importance in veterinary medicine. Cragg (1912) contributed several important papers on the mouth parts, especially those of the blood-sucking species *M. crassirostris* and allied forms. Since that early date my interest in the genus has slowly extended. Many more species were collected and studied in the field and the laboratory in different parts of the world. As my knowledge of the species extended it was soon realised that no further progress would be made towards establishing the true identity of many of the commonest species until all the existing types of the older authors were examined. This I was able to accomplish in 1922 and to clear up most of the synonymy. And in addition I then had the opportunity of studying large collections in Kiel, Stockholm, Copenhagen, Berlin, Vienna, Budapest, Turin and Paris and the National collection in London. I also worked through the collection of the Indian Museum and that of the Entomological Department of the Agricultural College at Pusa, India.

I would like to take this opportunity of acknowledging the assistance given me by the late Professor Bezzi, who for nearly twenty years helped me in every possible way with identifications and specimens. And I look back with pleasure to the time I spent with him in 1922 at Turin working through his collection. I would,

therefore, like to place on record here that any merit my work on the higher Diptera possesses is due entirely to Professor Bezzi's encouragement, early guidance and continued help. I would also like to acknowledge the help ungrudgingly given me by Major Austen for a number of years. And my thanks are also due to Dr. Villeneuve, who has helped me with many specimens and determinations. During the twenty-seven years that I have been studying the species of *Musca*, I have amassed a collection which must, I think, be unique in that it not only contains nearly all the adults of the known species, but also many early stages, and hundreds of mounted preparations illustrating the structure of the larvae, the mouth parts of the adults and the terminalia. And I cannot close my acknowledgments without expressing my grateful thanks to all those, and they are so many that space will not permit me to name them all, who have collected and sent me material from all parts of the world; without their help, this and the succeeding papers could not have been possible.

Those who have studied the species of *Musca* will, I think, agree that there are few genera of the Muscinae which present greater difficulties in the interpretation of the species and their relationships to each other. I have, therefore, long recognised the importance of discovering some character or characters, which would not only indicate the exact limits of the genus, but which would give a certain clue to the true affinities of the species. As far back as 1911 I examined the terminalia of several species, and still have the slides, but as I failed at that date to appreciate the true significance of these structures in separating the species, and as my technique was faulty, nothing further was done. To Awati (1916) is due the credit of elucidating the true nature of the terminalic appendages of *Musca*, and of showing that these structures afford useful characters for separating the species. As he did not, however, at the time know the species, he was unable to use the characters of these structures for separating them. He showed, however, that the male terminalia fall into several groups according to their structure, but he missed the true significance of the groups; he also failed to appreciate the importance of the paramere. Awati's paper has, unfortunately, either been missed, or ignored, by subsequent writers on the male terminalia of the higher Diptera, with the result that the

terminology used even by recent writers is not only inaccurate but misleading.

In collaboration with Miss MacGill (1925), I undertook a study of the antennae of the higher Diptera, paying special attention to the structure and arrangement of the sense pits, and it was discovered that, in all the species of *Musca* then examined, the third antennal segment has a large, basal, grape-like sensorium opening by a duct into the socket. Since then the antenna of all the remaining species has been examined, and it is now known that this sensorium is present in the third segment of every species, and in no other higher Dipteron. This character is, therefore, of fundamental importance in showing that the genus is a homogeneous one, the species being very closely related to each other. The early stages, too, exhibit several characters in common, such as the shape and structure of the posterior spiracles of the larva and the existence of a small, short, left oral hook. Fundamental as these characters are, they do not, however, give us any clue to the evolution of the species in the genus, nor do they help us to understand the status of many of the apparently specialized species, such as the blood-sucking and larviparous forms, and their relationships to each other and to the simpler ones in particular.

In 1922 I made another attempt to study the terminalia in the light of Awati's work, and came to the conclusion that these structures do not afford any reliable characters for separating the species. This statement was published in my papers on the Oriental (1924), and again in the paper on the Ethiopian species (1926). Since then I have re-examined the preparations then made and can well see how this erroneous conclusion was reached. In the first place my technique was again faulty, the parts being mounted flat in different directions. Further, the study was limited to the genus *Musca*, and to the terminalia of some species of one group only, in which the differences are small and difficult to detect without some knowledge of allied forms. I failed to study the terminalia of species of allied genera, and so was not in a position to appreciate the true significance of these structures in elucidating their relationships. In 1930-31 I decided to make a third attempt but this time, before studying the terminalia of *Musca*, I first worked through those of all the other genera of the Muscinae, especially of all the

species of *Glossina*. The specimens, after being macerated, were cleared, dissected off, and then studied in clove oil in a solid watch glass with the aid of needles, a high-power binocular microscope and a good light; whenever necessary rough drawings were made. The various parts were then dissected off and mounted on slides in Canada balsam in the same direction and *without compression*. Each was then drawn to the same scale, so that all the illustrations were strictly comparable. After studying all this material from the comparative standpoint, and especially comparing all the drawings, the male terminalia of all the known species of *Musca* available were similarly prepared, studied and drawn to the same scale. How completely the work has been done will be appreciated by the fact that more than six hundred microscopic slides have now been made of the parts, each mounted in the same way and without compression. And more than three hundred drawings of the parts of the male terminalia of *Musca* have been executed. It is now possible to confirm Awati's conclusions that the terminalia of *Musca* afford valuable characters of use in separating and finally establishing the identity of the species. And it is possible to go further than this, and to say that the characters of the male terminalia also provide us with the only means of understanding the true nature of such apparently specialised forms as *M. albina*, *M. planiceps*, *M. inferior*, *M. senior-whitei*, *M. fletcheri* and *M. crassirostris*. Further, the comparative study of these structures has enabled me to arrange the species into three groups according to their affinities.

Before giving a short description of the segmentation of the abdomen of the male *Musca domestica* and the terminalia, it is necessary to explain the meaning of this term. It is now well known that the terminal abdominal appendages of male insects afford not only valuable taxonomic characters for separating closely allied species, but, when studied from the comparative standpoint, help us to understand any given species, no matter how anomalous it may be in other respects, and also provide us with a means of grouping species into genera, and into groups within each genus, according to the true affinities of the species. The importance of these embryonic structures in the higher Diptera is noted by Schnabl and Dziedzicki (1911) in their work on the Anthomyidae, when they say in the opening sentence of their preface: 'Die natürliche

Gruppierung der Anthomyiden-Arten ohne spezielle Kenntnis und Berücksichtigung der männlichen Kopulationsorganoide ist ungemein schwierig und selbst in vielen Fällen unmöglich.' In the case of *Musca* it is quite impossible to group the species according to their relationships on characters other than the male terminalia; this fact will be conclusively and finally proved in this paper.

These terminal abdominal appendages are usually referred to as the genitalia, genital apparatus, genital armature and hypopygium. As pointed out by Freeborn (1924), the first three terms exclude the anal segment and its appendages, and they are, to say the least of them, clumsy expressions, and it is sincerely hoped they will be dropped. The last, which means the under rump, is used either loosely for the genital segment and its appendages, for the anal segment and its appendages, or frequently for both; some authors illustrate only the anal cerci and refer to them as the hypopygium. In view of this most unsatisfactory terminology, Freeborn introduced the word terminalia, which I agree aptly refers to the whole complex of genital and anal segments and their appendages. This term is a much more appropriate one, and especially so in the case of the higher Diptera, for it is the characters of the anal (terminal) segment, and its appendages which are so commonly used in this specialised branch of systematic Dipterology, and it is inaccurate to refer to it, as is very frequently done, as the genitalia. I have, therefore, adopted this term and will use it throughout my papers and books. By the word terminalia is meant, then, the terminal abdominal segments and their embryonic appendages which go to make up the genital and anal armature. These are, in the higher Diptera, the anal cerci (appendages of the obsolete eleventh segment), the tenth segment mainly its tergum, the ninth or genital segment with its appendages the coxites, and the phallosome and parameres, and the sternum of the fifth segment and its lateral processes. Some systematic Dipterologists appear to be under the impression that terminalic characters are merely those of a single organ, the penis, and they argue that chaetotactic and pilotactic characters are of equal, if not of greater value, as they are to be found all over the body and are probably of phylogenetic significance. It is necessary to emphasise the fact that terminalic characters are not those of a single organ but of parts of three segments (and sometimes more),

and their embryonic appendages, structures which beyond any doubt have a deep significance, and take us back to the past history of the species. I cannot believe that the presence or absence of a single bristle, the curvature of bristles, the presence or absence of hairs, etc., have any phylogenetic meaning in these flies.

As the illustrations of the parts, especially the phallosome, paramere, anal cerci and fifth sternum, are all drawn to the same scale by myself with the aid of a camera lucida, and are drawn from specimens which have been mounted *without compression*, they are, therefore, as accurate as it is possible to make them; they will last as illustrations of the parts as long as the blocks themselves last. In order that they may remain of use as long as possible, I have, where lettering is used, numbered with Arabic numerals those parts the terminology of which may change with increasing knowledge. If, perhaps, after ten years time, the terminology of a part changes, all that will be necessary will be to alter it in the legend to the drawing, which will still remain accurate. In most cases I have drawn the terminalia of specimens from type localities, and in many cases from type material, thus ensuring still further the utility of the illustrations. A careful examination of the drawings of paired structures, anal cerci for instance, will demonstrate that they are never exactly alike, one is usually smaller than the other, and may even be differently shaped; this and other peculiarities have been brought out in the drawings.

SHORT DESCRIPTION OF THE MALE TERMINALIA IN *MUSCA*

Awati (1916), in the paper already referred to, made a comparative study of the male terminalia of both the lower and higher Diptera, and in order to interpret the parts took advantage of certain clues afforded by a study of macerated specimens of the entire abdomen of the male; he noted particularly the following:—

- (1) The number of spiracles in the abdomen.
- (2) The position of the anal opening.
- (3) The attachment of the theca (penis).

As a result of his studies he came to the following conclusions:—

- (1) The eighth or pregenital segment has disappeared in the higher Diptera.
- (2) The genital segment is the ninth; it has no spiracles and its sternum has disappeared.
- (3) The tenth or anal segment also has no spiracles, and is now only represented by the anal cerci which are its appendages.

After a long comparative study of the male abdomen in some lower and in many higher Diptera, and especially that of *Glossina*, *Gasterophilus*, *Oestrus*, *Cephalopsis*, *Sarcophaga*, etc., I have come to the conclusion that Awati's interpretation of most of the terminalic structures is as near the truth regarding their true identity as it is possible to reach, short of a study of the development of the parts. Awati, in this paper, established two important facts, viz. : (1) In the higher Diptera the eighth abdominal segment has entirely disappeared, and that (2) the appendages attached to the end of the anal or tenth segment in the male are the anal cerci, and are homologous with the corresponding structures at the end of the female abdomen. It is unfortunate that most subsequent writers who have had occasion to refer to, or describe, the terminalia of any of the higher Diptera, still speak of the eighth segment, and thus arrive at erroneous conclusions regarding the segmentation of the abdomen. And the anal cerci are not recognised as such, but are referred to by a host of names, such as superior claspers, mesolobes, superior forceps, obere Zange, mesocerci, eighth sternite, lamina genital, etc., all terms which have no meaning in most cases. With regard to the ninth or genital segment and its appendages, Awati came to the conclusion that the sternum had disappeared and that the appendages of this segment were modified to form claspers. It matters little whether or not these interpretations are entirely correct, as long as the true significance of these terminalic characters are clearly recognised in systematic work, and such terms are used which appear to be more in keeping with the homologies of the parts as known at present. Once the true significance of the parts is recognised in tracing the evolution of species in genera, and groups as a whole, towards still higher forms, then it becomes possible to elaborate a true grouping or classification according to relationships. I will now give a short account of the segmentation of the male abdomen in *Musca* and then describe briefly the terminalia. This description has been drawn up from the study of many abdomens dissected off from the metathorax and examined in clove oil with a binocular microscope, needles and a good light. The terminalia are described from a study of the parts in clove oil and then mounted in Canada balsam on slides without pressure.

SEGMENTATION OF THE MALE ABDOMEN IN MUSCA (Fig. 1). The abdomen of the male *M. domestica*, as seen from the dorsal surface, appears to consist of four visible segments. The apparent first tergum, however, is clearly a compound sclerite consisting of the

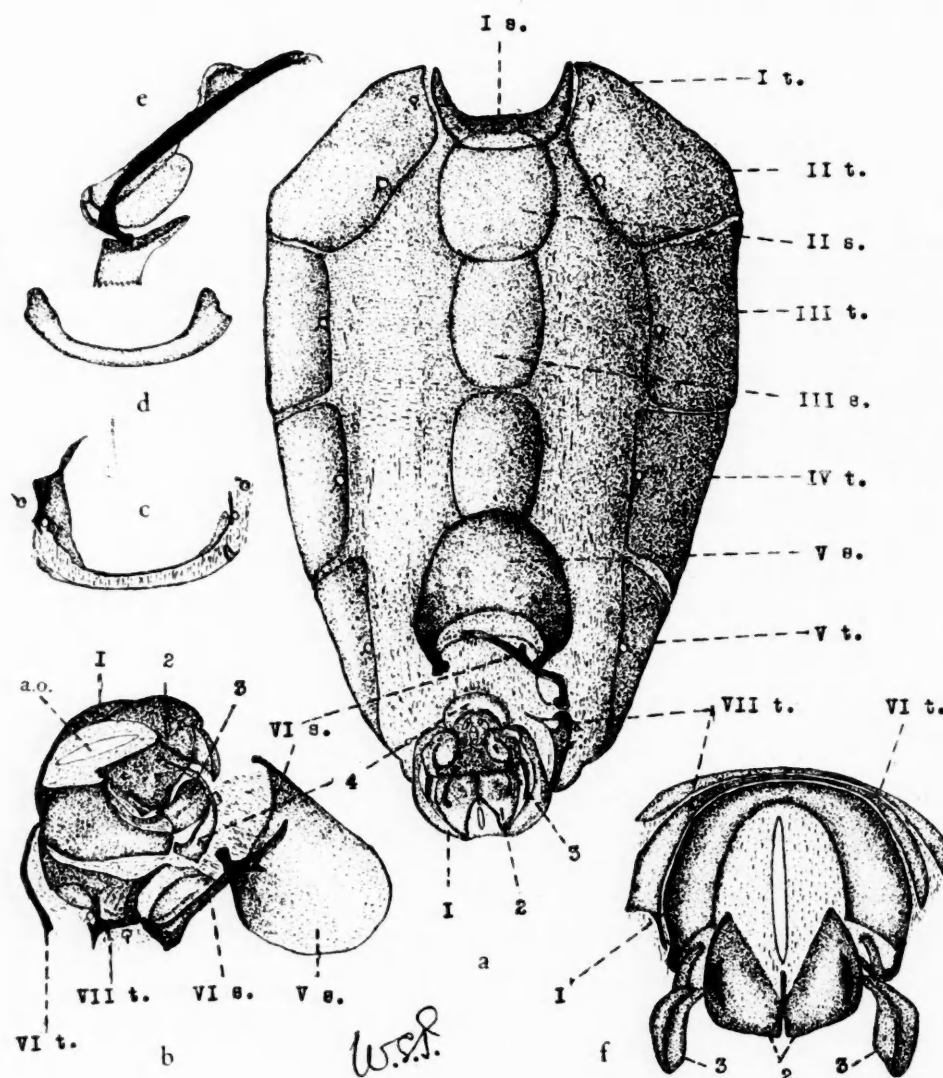


FIG. 1. a.—Ventral view of abdomen of ♂ *M. domestica*. Is., IIs., IIIs., Vs., VIs., VIIs., VIIs., VIIs.—1st, 2nd, 3rd, 5th, 6th sternite; It., II t., III t., IV t., V t., VI t., VII t.—1st, 2nd, 3rd, 4th, 5th, 6th, 7th terga; 1—Xth and possibly part of XIth tergum much reduced; 2—anal cerci; 3—coxites of IXth segment; 4—IXth tergo-sternum—note how close the spiracles are to the edge of their respective terga. b.—♂ terminalia of *M. domestica* in side view. a.o.—anal opening; other lettering as in a. c.—VIth, VIIth terga, showing position of 6th, 7th spiracles. d.—VIth tergum, showing anterior prolongations. e.—Articulation between VIIth tergum and VIth sternum. f.—Front view of ♂ terminalia of *M. domestica* showing anal cerci and IXth coxites in extended position, lettering as in a.

reduced first tergum, which has become fused with the larger and more normal second tergum. The proof of this lies in the fact that this compound segment possesses two spiracles and two sterna, and it is well known that there is practically no reduction in the number

of the spiracles, or the sterna, at the base of the abdomen. The posterior limit of the first tergum may be defined by the fringe of rather long, projecting hairs on each latero-ventral border; a line joining the hairs across the dorsal surface of the compound tergum would approximately mark the posterior border of the first true tergum. The first spiracle is situated at the edge of the first tergum a little distance from its ventro-median margin, at about the level of the first sternum, which is narrowly U-shaped, and is very commonly dark brown, as is also the true first tergum. The second sternum is a large, oval sclerite. The second spiracle is situated at the ventro-median edge of the second tergum about the level of the middle of the second sternum. The third tergum is long, and is about the length of the compound first and second terga; in *domestica* and commonly in other species, it has a broad, median, dark stripe. The third sternum is narrower and more rectangular in shape than the first. The third spiracle is situated at the very edge of the third tergum a little posterior to the middle of its ventro-median margin. The fourth tergum is slightly shorter than the third, and is about equal in length to the compound first and second. The fourth sternum is very similar to the third. The fourth spiracle is situated on the edge of its tergum about the middle of the ventro-median border. The fifth tergum (apparent fourth) is the last visible dorsal sclerite. It is about as long as the fourth, and narrows a little posteriorly where its sides are rounded. Tucked away ventral to it are the modified terminalic segments (fig. 1, a). The fifth sternum is modified to form a large, somewhat triangular or rectangular plate, the anterior end rounded, the posterior margin emarginated, and the lateral ends produced to form short or long processes, often dilated distally, and commonly serrated; these processes are the so-called primary forceps of authors. Whether they represent the remains of true coxites of the fifth segment seems doubtful. In this and in all subsequent papers, they will simply be referred to as the lateral processes of the fifth sternum. They afford some useful subsidiary characters in identification of genera, and to a less extent species; it should be clearly noted that in *Musca* the lateral processes vary in shape, length and amount of serration in the same species, and in the same specimen on the two sides. The posterior margin of the fifth sternum with its

lateral processes forms the anterior lip to the genital sinus or atrium (fig. 1, *a*, *b*). Strictly speaking, it has nothing to do with the terminalia, but has come into close association with the genital segment as a result of the reduction of the sixth and seventh sterna and the complete absence of the eighth segment.

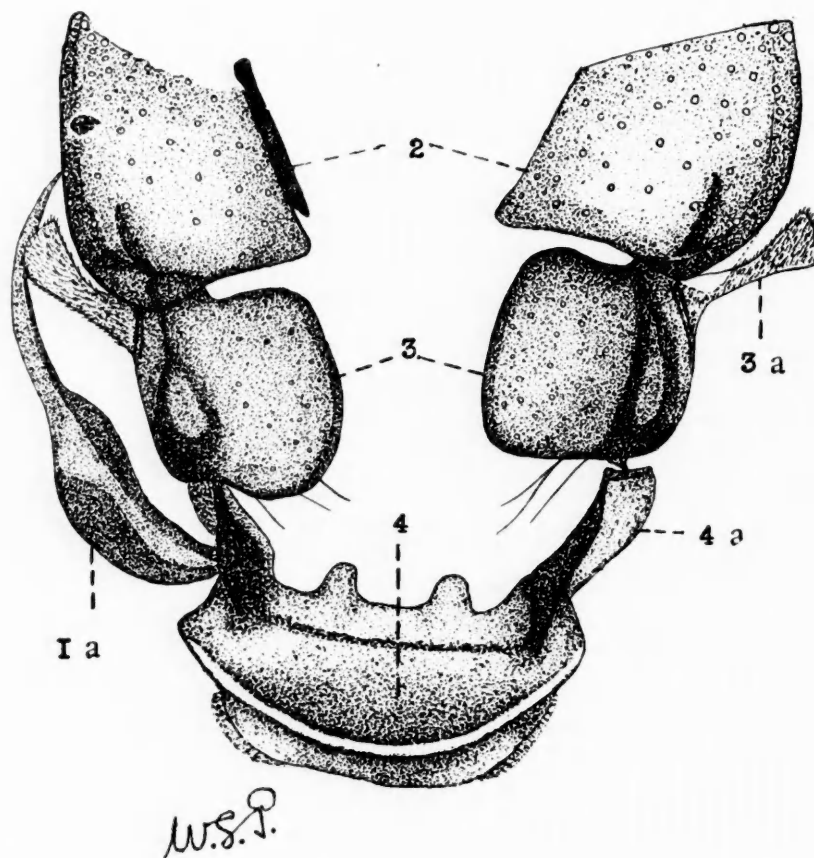


FIG. 2. IXth tergo-sternum of ♂ *Musca domestica* showing articulation with its coxites, and these again with the anal cerci which are shown separated; the phallosome and parameres have been dissected off. 4.—IXth tergo-sternum; 4a.—lateral process articulating with coxite; 1a.—process of Xth tergum articulating with lateral process; 3.—coxites of IXth tergo-sternum; 3a.—part of coxite possibly basal segment; 2.—anal cerci separated, note the wedge which joins them. Note the small raised processes on each side of the posterior border of the IXth tergo-sternum to which the parameres are attached.

The sixth tergum (fig. 1, *d*, *f*) is a narrow transverse strip of chitin lying under the edge of the fifth tergum, and is about half its width; it is expanded at both sides and produced anteriorly into short processes (fig. 1, *d*). The sixth sternum is asymmetrically developed, the left side only being present, the right being replaced by membrane (fig. 1, *a*, *b*). It consists of an irregularly shaped, rod-like sclerite, expanded and bent at its junction with the seventh tergum; it extends round towards the middle line as a rod-shaped

bar. At its outer end just below its articulation with the seventh tergum, it has a thin, plate-like expansion directed backwards and slightly inwards (fig. 1, *b, e*); it gives attachment to the membrane which probably represents the obsolete seventh sternum. The seventh tergum is also asymmetrical and is more strongly developed on the left side (fig. 1, *f*). It is longer and wider than the sixth tergum though similar in general structure, and from the dorsal side is seen as a thin curved plate. On the left side it expands into a somewhat triangular structure with a marked antero-dorsal projection, which is emarginated to accommodate the seventh spiracle (fig. 1, *c*). In some species the spiracle is situated on the tergum itself. The sixth spiracle is located in the membrane, anterior and close to the base of the expansion (fig. 1, *c*). The ventral edge of the tergum is slightly hollowed out for the articulation of the sixth sternum (fig. 1, *e*). The spiracles on the right side are located in the membrane (fig. 1, *c*). As already noted, the seventh sternum is entirely membraneous and is attached anteriorly to the sixth sternum and posteriorly to the ninth tergo-sternum. The eighth segment has entirely disappeared.

TERMINALIA PROPER. NINTH SEGMENT (Figs. 2, 3). The ninth segment has become invaginated into the end of the abdomen in the genital sinus between the seventh and tenth segments, and it lies concealed on the ventral side (fig. 1, *a, b*). It bears the penis and the parameres, and has attached to it a pair of appendages, the coxites (fig. 2). It is well protected from injury by the fifth sternum and its lateral processes, and by the sixth sternum on the left side (fig. 1, *a, b*). Ventrally and posteriorly it is protected by its coxites and the anal cerci (fig. 1, *a, b*). It consists of a single median sclerite, the anterior surface of which is rounded and semi-circular in outline in *domestica* (figs. 2, 3); at rest it lies almost in the vertical plane. There is some evidence that it represents the fused tergum and sternum of the ninth segment; Awati, however, considers that the sternum has disappeared. For convenience, this single sclerite will be referred to in this and in succeeding papers as the ninth tergo-sternum, and is lettered 4. Its rounded dorsal surface or edge is thickened and projects antero-ventrally as a lip, which is much better developed in some species than in others (fig. 3, *a*). The seventh sternum, or what appears to be located in its position, and

which as already noted is membranous, is attached posteriorly along the dorsal margin of the ninth tergo-sternum; anteriorly it is attached to the sixth sternum. This membrane forms a very efficient pocket in which the long penis of the Calliphorinae can rest and thus be protected from injury; it affords some protection too

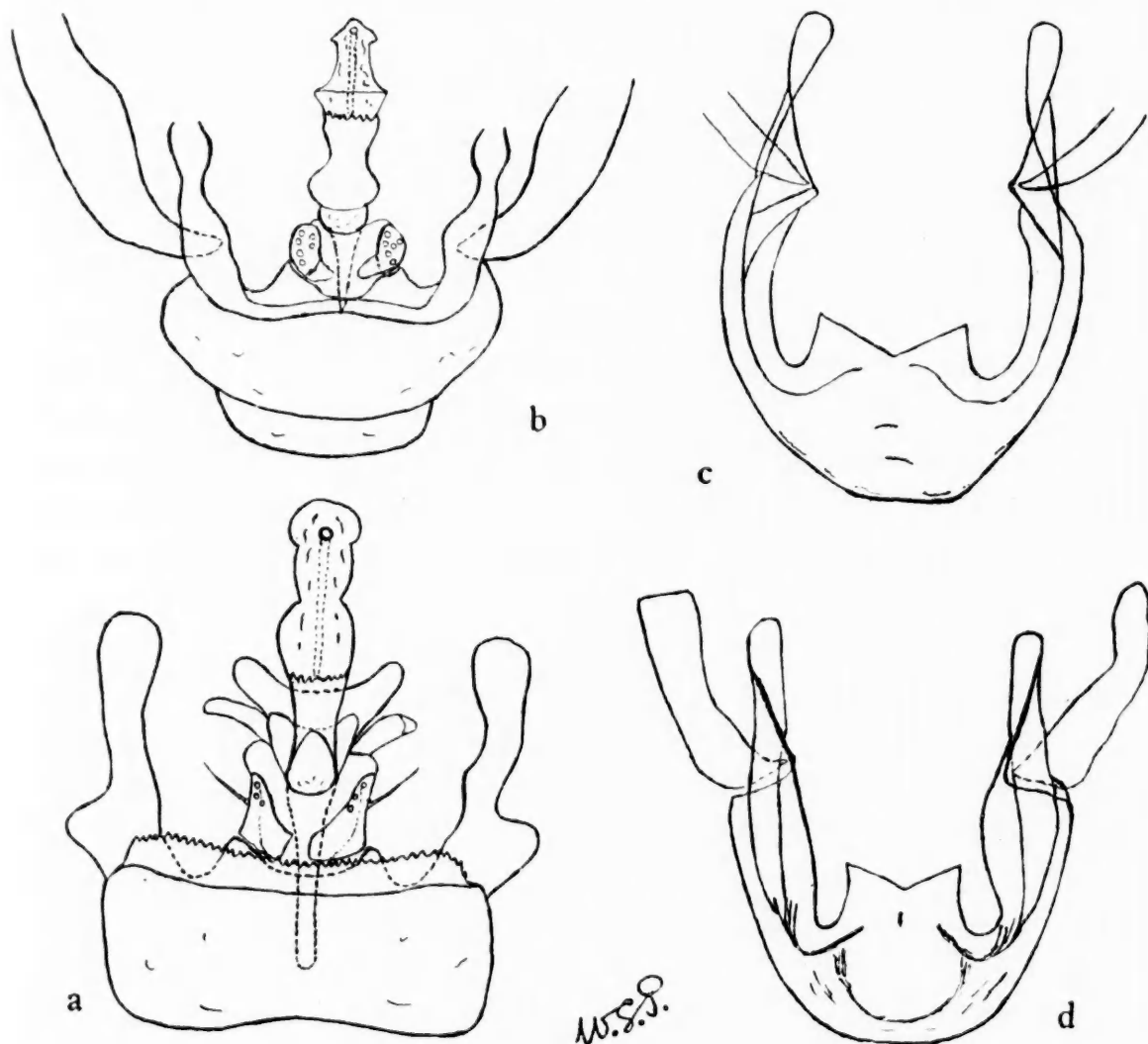


FIG. 3. *a*.—Front view of IXth tergo-sternum of *M. lusoria*—note its rectangular shape, the anterior lip with its serrated margin, note the anterior part of paramere bearing bristles, and the posterior part projecting at side, also note the large, hood-shaped, Y-forked posterior process, and lastly the body of the phallosome rather foreshortened, the membranous part well seen. *b*.—Front view of IXth tergo-sternum of *M. domestica*—note the small anterior part of paramere, the posterior part not visible, the posterior process is also hidden behind the body of phallosome. *c*.—IXth tergo-sternum of *M. gabonensis*. *d*.—Same of *M. gibsoni*. Compare the shape of these two with those of *domestica* and *lusoria*.

to the short penis in *Musca*. The ventral margin of the ninth tergo-sternum is also thickened and strengthened, and has two raised processes on each side of the middle line (figs. 2, 3). It is on these projections that the small parameres at the base of the penis rest,

the penis being attached to the margin in the hollow between them (fig. 3, *a*, *b*). On each side the posterior border is extended into two long processes, the chitin twisted to ensure strength, as is so characteristic of apodemes, etc. (fig. 2, 3, 4 *a*). Each process articulates through rather a loose membranous joint with the antero-dorsal edge of the coxite of the ninth segment (figs. 2, 4 *a*). The ninth tergo-sternum, though of uniform structure in *Musca*, varies a good deal in shape (fig. 3). The outer process of the tenth tergum fits by a blunt pointed process into a socket about the middle of the outer side of the lateral process of the ninth tergo-sternum (fig. 2, *a*).

PHALLOSOME (fig. 4). The term phallosome was introduced by Christophers and Cragg (1921) for the intromittent organ in *Cimex*. They pointed out that 'No satisfactory term to indicate the complicated non-segmental intermittent organ as a whole appears to exist. Sharp dealing with the male organ in the Pentatomidae speaks of the *aedoeagus*, but apparently does not include in this term the *theca*, an essential and important part of the non-segmental mass. Edwards uses *aeodeagus* for the parts in the mosquito but the same term is used in a restricted sense in the Lepidoptera. As it is often convenient to signify the whole non-segmental mass without implying homologies other than in the organ as a whole, we have used the term *phallosome*, which is in keeping with the nomenclature now coming into general use and is devoid of ambiguity.' I propose adopting this term in the future to indicate the entire intromittent organ in the higher Diptera without implying any homologies. Fig. 4 is an outline drawing of the phallosome and one paramere of *Musca domestica*, drawn from the side, after being dissected off from its attachment and mounted on a slide without compression. It illustrates how these structures in the case of all the species have been drawn to the same scale, so that no difficulty should be experienced in recognising the parts in the drawings although no lettering is used. Around each phallosome, there are, in most of the illustrations, enlarged drawings of the parts seen in different positions and from specimens from different localities; but it should be noted that these additional illustrations have not been drawn to the same scale as has the phallosome and paramere in each species, and they are, therefore, not comparable. *a* is regarded as

the body of the phallosome, and it consists of a chitinous and membranous part; *b* is the shaft or apodeme; *c* is the posterior process; *d* 1 is the anterior part of the paramere; *d* 2 is the posterior part. In each case the phallosome is in the erect position, its anterior face is directed to the right.

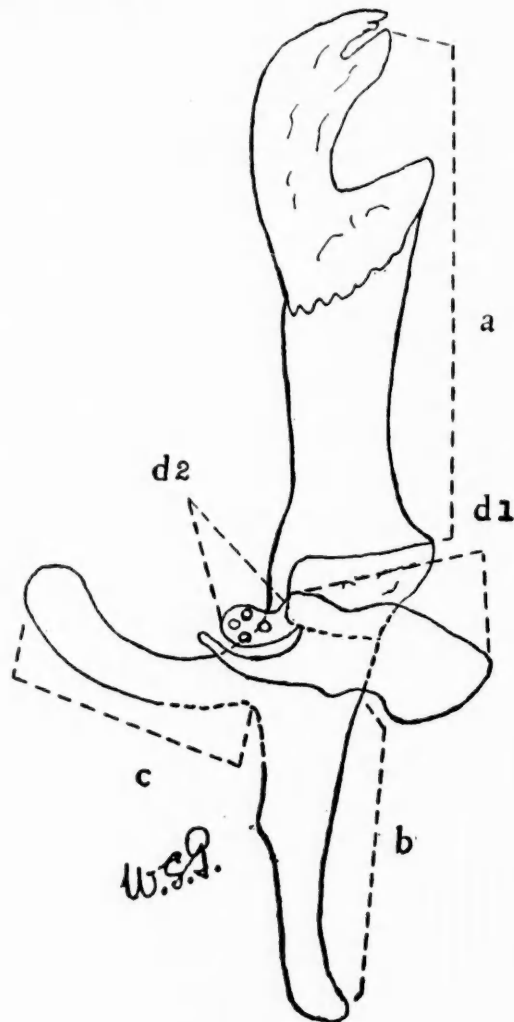


FIG. 4. Phallosome and one paramere of *M. domestica* to show its structure; its anterior face is directed to the right. It is illustrated in this position and to the same scale in the case of all the species. *a*.—body of phallosome—note its two parts, chitinous and membranous; *b*.—shaft or apodeme; *c*.—posterior process; *d* 1.—anterior part of paramere; *d* 2.—posterior part of same, note its size and the four round bases of sense spines.

The phallosome lies in the middle line dorso-ventrally. It is flanked on each side by a small, raised, chitinous structure here regarded as the paramere. The body is a simple tube partly chitinous and partly membranous; it has no chitinous adornment such as the various gadgets to be seen on the phallosome of the Calliphorinae, and especially the Sarcophaginae. And it should be specially noted that it is uniform in structure in every one of the forty-one species

studied. The two parts vary in length in the different species, but in most the chitinous part is, if anything, the longer. Proximally the chitinous part is continued posteriorly into a characteristic bent, often lightly chitinized, process, which in the resting position is

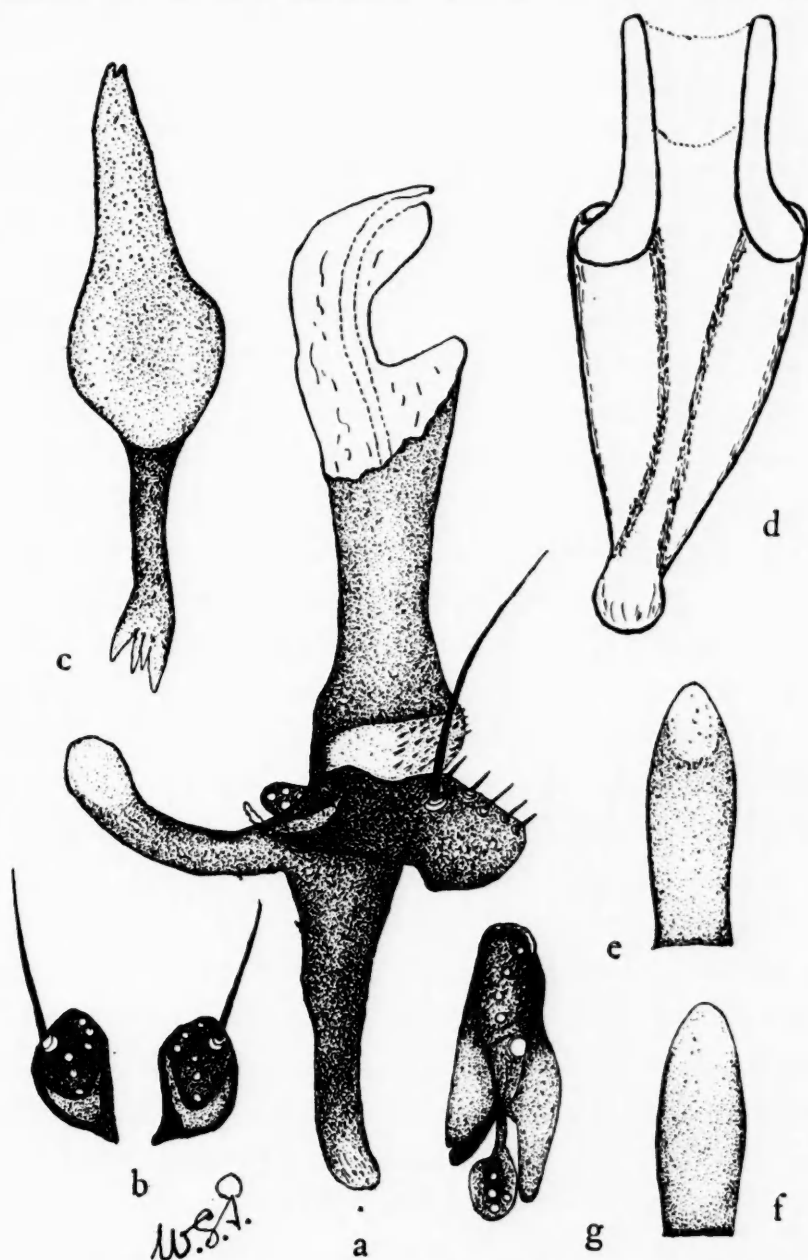


FIG. 5. *a*.—Phallosome and one paramere of *M. domestica*. Note the bristles on the anterior part of paramere, and the small almost flat posterior part with its sense spines; *b*.—anterior part of paramere seen from front; *c*.—sclerite of sperm pump; *d*.—apodeme of phallosome seen from front; *e*.—posterior process of phallosome seen from front—note that it is not expanded; *f*.—another view of same; *g*.—paramere seen from above—note the shape and relative size of the two parts, especially the posterior.

directed backwards and slightly ventrally, its concave face being directed towards the phallosome anteriorly. This process is of importance in affording a subsidiary character for grouping the

species ; it will be referred to in this and subsequent papers as the posterior process of the phallosome. It is extremely characteristic of the genus *Musca* being present in all the species, and in the lower forms is a simple process, but becomes finally expanded into a large, hood-like, Y-forked structure in the highest species. It is absent in some genera of the Muscinae, notably in the *Stomoxys* group and in all the species of *Glossina*. It is present in many of the genera of the Calliphoridae. It is a simple process in *domestica* and its allies. The phallosome is continued postero-dorsally (depending on the position of the organ) into a strongly chitinized shaft or apodeme, which, in side view, appears like a curved rod (figs. 4, 5), but when seen either from the front or from behind (figs. 5, *d*, 26, *e*), is seen to be a narrow plate widest nearest the phallosome and narrowing distally into a blunt, rounded or square process which is commonly turned forwards. This surface is directed anteriorly, and is strengthened by two bars which converge towards the apex. The end nearest the phallosome also has two strong bar-like side pieces which strengthen the body at the level of the posterior process. This structure will be referred to as the apodeme or shaft of the phallosome (fig. 4, *b*) ; it may possibly be homologous with the so-called basal plates in the terminalia of the mosquito. The phallosome is jointed to the shaft by a membranous part, which is commonly covered with fine spines and which permits of the organ being flexed and erected. The ejaculatory duct after leaving its sclerite, passes into the phallosome between the posterior process and the shaft, and extends up through the chitinous portion into the membranous part, to end in the middle line at the distal end. The sclerite (fig. 5, *c*), of the ejaculatory duct is of considerable size in *Musca*. The duct is attached to it and is much enlarged to form a pump, the sclerite giving origin to numerous muscle fibres, which extend over and around it, and, compressing it, not only draws the seminal fluid into the sac, but drives on what is in it into the distal part of the duct in the phallosome.

PARAMERE (figs. 4, 5). As already noted, there is on each side of the phallosome, about the level of the posterior process, a small, elongated, raised, chitinous structure which is attached by membrane to the side of the phallosome, the back of the posterior process, the lateral processes of the ninth tergo-sternum, and the bases of the

ninth coxites ; the membrane separates the anal compartment from the genital. Each paramere rests against the raised process on each side of the middle line of the ventral border of the ninth tergo-sternum, to which it is attached. When dissected off and mounted in different positions, and examined with an oil immersion lens, it will be noted that each is a small plate of chitin bent round to form a hollow structure closed anteriorly, the sides parallel and ending posteriorly in two, long, curved, rather narrow processes, which enclose posteriorly a small, slightly raised, round, chitinous plate (figs 4, 5, g). Each paramere lies in the antero-posterior plane at right-angles to the phallosome. Its two parts can easily be recognised in a dorsal view (fig. 5, g). The anterior part is much larger and longer than the posterior in the group to which *domestica* belongs ; when seen from the front it appears rounded and narrow. It always bears a number of bristles, usually one long and several smaller ones, the latter commonly arranged in two lines but often they are irregularly placed, and most are on the outer sloping surface. The number and arrangement of the bristles vary in the same species, and even in the two parameres of the same specimen so that no two are exactly alike ; there is, however, in most species a definite number and arrangement. In *domestica* there are from five to seven bristles, one always longer than the others (fig. 5, a, b, g). It should be clearly understood that, like hairs and bristles elsewhere, these are variable and of minor importance. The shape of the anterior part varies according to the position from which it is viewed.

The posterior portion (figs. 4, d, 2, 5, g), which is attached in *domestica* by a rather narrow slip of chitin to the anterior part lies in the fork or emargination produced by the two posterior processes of the anterior part (fig. 5, g). In *domestica* and allies it is hardly raised and consists of a small, round, button-like plate and has four to six round, clear areas which are the bases of minute sense spines. In some species allied to *domestica*, the point of attachment to the anterior part appears to form a hump, giving the area a conical shape, and rather suggesting the shape of the posterior area in the *sorbens* group, but this appearance is merely due to the angle at which the area is viewed. In its simplest form then this part of the paramere is a small disc-shaped plate ; in the intermediate group (*sorbens*) it is much better developed, and forms a distinct cone-

shaped projection varying in height and width in the different species. In the third group, containing the most highly developed forms, the posterior process has grown into a long, bent process, and in this group as in the intermediate one the posterior part always bears the same characteristic small sense spines. The evolution of this part of the paramere in the three groups is illustrated in fig. 9.

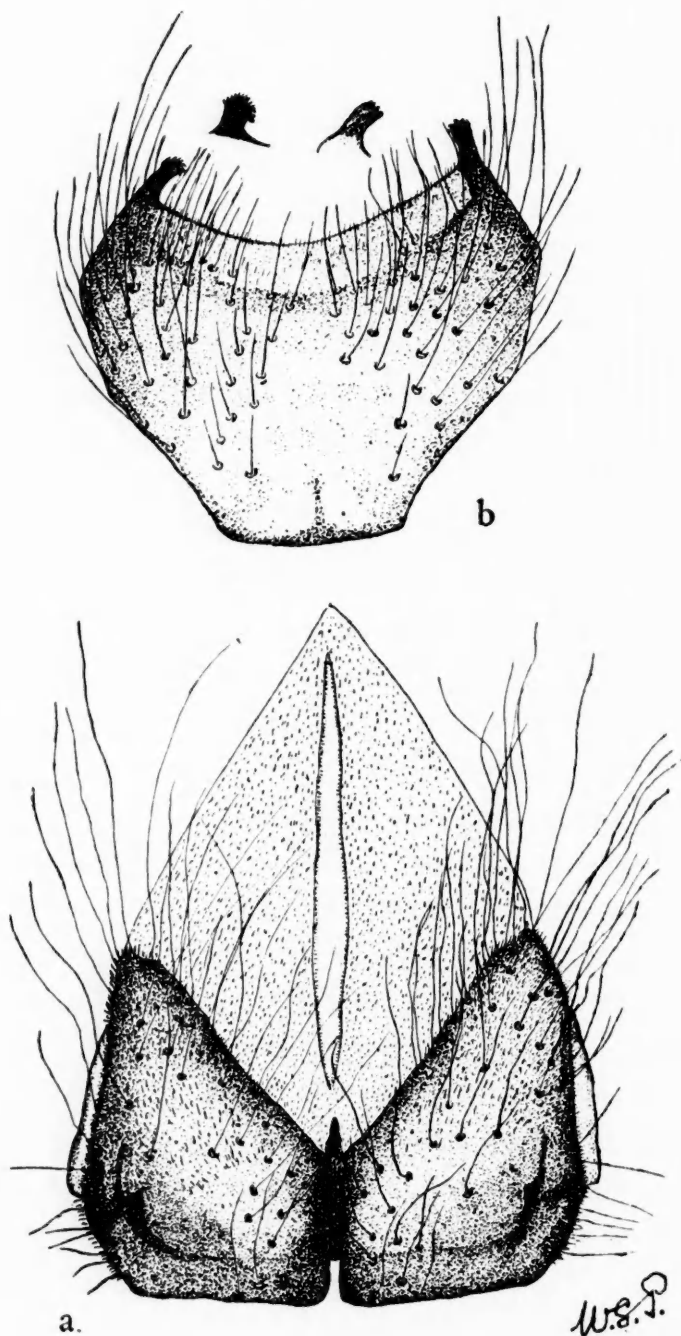


FIG. 6. *a.*—Anal cerci of *Musca domestica*—note there is no suggestion of nipple-like processes; *b.*—Vth sternum—note how it narrows posteriorly.

TENTH TERGUM (fig. 1, *b, f*). The tenth or anal segment consists of a markedly convex plate, the tergum, the convexity directed posteriorly and forming the apex of the abdomen. It is deeply incised postero-ventrally by a heart-shaped incision which does not extend the whole length of the tergum. The incision is closed by a stout membrane which is attached all along the margin. Extending dorso-ventrally almost the whole length of the membrane there is a narrow slit, the anal opening (*a.o.*), which is directed ventrally. The anal membrane is covered externally with fine spinulae. At its anterior end the incision is closed by the anal cerci, and each side of the tergum, after ending in a point, slopes outwards, and joining the anterior margin forms a long, curved, and twisted, rod-like plate, which ends in a blunt point in a socket at the side of the posterior process of the ninth tergo-sternum as already noted above (fig. 2, 1 *a*). This attachment permits of the ninth tergo-sternum swinging in an antero-ventral line. The convex posterior part of the tenth tergum bears many long bristles and hairs. The tenth sternum has either disappeared or is represented by the membrane which divides the cavity enclosed by the tergum, thus separating the anal compartment from the genital. The membrane is attached to the coxites of the ninth segment and the base of the posterior process of the phallosome.

ANAL CERCI (figs. 6, 7, 8). Attached by membrane to the inner end of the sloping posterior margin of the tenth tergum (see fig. 1, *f 2*), there is on each side a triangular plate, the two united along their inner borders rather loosely by a small slip of chitin; these plates are the anal cerci (fig. 6, *a*). They lie anterior to the anal membrane, their free ends directed antero-ventrally protecting the genital atrium. Each cercus is convex posteriorly. The long sloping inner margin is well supplied with long strong bristles, and is attached to its fellow by the anal membrane. The outer margin is rounded and is attached by membrane to the outwardly sloping inner margin of the tenth tergum (fig. 1, *f*). At the junction of the two margins, each cercus hinges against the tenth tergum at the anterior end of the emargination, and before it slopes outwards. In the membrane, between the outer margin and the margin of the tenth tergum, there is a small flat sclerite covered with numerous hairs, which is part of the ninth coxite. The anterior or free margin of each cercus varies in shape according to the species. In the simpler forms this margin

slopes from the middle line outwards, the outer and inner ends being more or less rounded, especially the former (fig. 6, *a*). Along the inner margin of the anterior end, as already noted, each cercus is loosely attached to its fellow; the two can easily be separated by light traction. The end of the inner margin tends to develop into a nipple-shaped process in some of the species of the intermediate group (*sorbens*), and becomes a well-marked projecting process in the

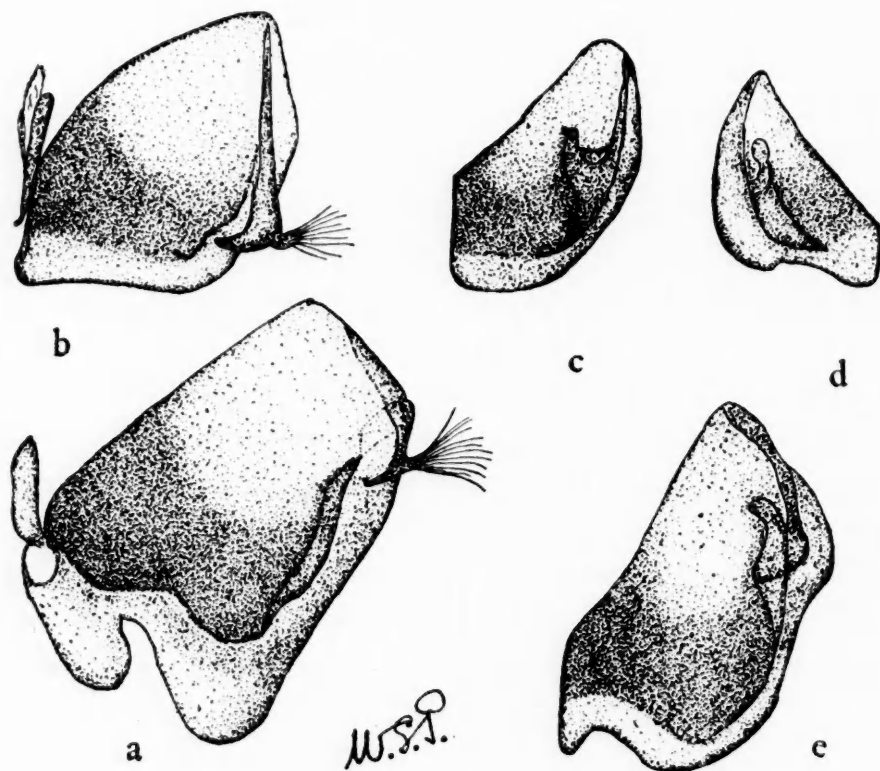


FIG. 7. *a*.—Ventral view of one anal cercus of *M. lusoria*—note the two nipple-like processes, the point of articulation of IXth coxite is located towards the posterior end; *b*.—ventral view of one anal cercus of *M. domestica*—note its characteristic shape and the anterior position of the articulation with the IXth coxite; *c*.—ventral view of anal cercus of *M. terrae reginae*—note the very rounded and sloping outer end; *d*.—ventral view of anal cercus of *M. tempestiva*—note the suggestion of a process forming at both ends, the space in between being emarginated; *e*.—ventral view of anal cercus of *M. gibsoni*—note the well-marked nipple at the inner, and a suggestion of one at the outer, end.

species of the *lusoria* group. On the inner convex face of each cercus along the outer border there is a strong infolding of chitin, which provides surface not only for muscle attachment but for the articulation of the coxite of the ninth segment (figs. 7, 8). The characteristic wing-like shape of the two cerci in *Musca* is well seen in the illustrations (figs. 1, *f*, 6, *a*).

COXITE OF THE NINTH SEGMENT (fig. 8). The last structure to be described is a characteristic, rectangular sclerite which lies on each side, anterior to the free margin of the anal cerci (fig. 1, f 3); together they form the posterior boundary of the genital sinus. Each sclerite is shaped somewhat like the human hand, minus the thumb and fingers, and consists of two distinct plates of chitin fused together in the middle line but separated above; each of these sclerites is convex externally and concave internally. The free margin of each is directed towards the middle line where they almost meet, and in the normal resting position lie dorso-ventral; these structures will be

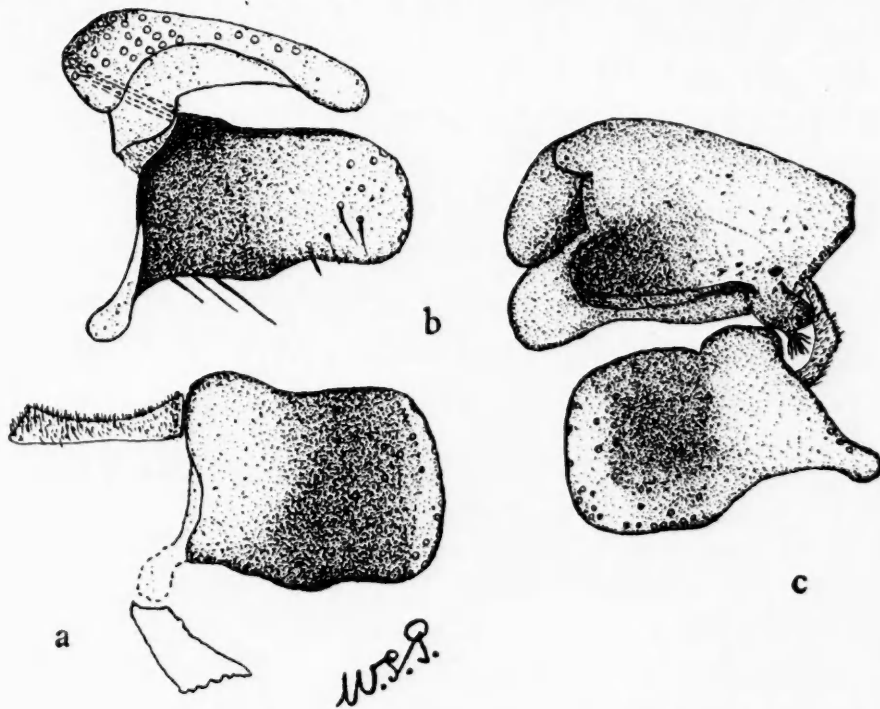


FIG. 8. *a*.—Outer surface of IXth coxite of *M. domestica*, showing characteristic shape, the hairy basal sclerite and articulation with the lateral process of IXth tergo-sternum; *b*.—same, showing its articulation with the anal cercus—note the hairs and bristles on the inner face, especially those near base; *c*.—anal cercus and IXth coxite, *M. lusoria*, also showing method of articulation.

readily made out with the aid of the drawings (figs. 3, 8). Dorsally and on the external side, the two parts of each sclerite separate, and the inner forms a broad sheet which lies ventrally. The dorsal edge of it articulates with the posterior process of the ninth tergo-sternum, and the ventral with the thickened margin on the convex side of the outer end of the anal cercus of its side (figs. 3, 8). The outer part of each coxite narrows, and is joined to a flat, hairy plate, which lies in the membrane between the outer edge of the anal cercus and the sloping anterior margin of the tenth tergum (fig. 2, 3*a*). These

appendages are then articulated, on one hand with the cerci, and on the other with the ninth tergo-sternum. They are commonly referred to as the inner forceps, forcepes inferiores, paralobes and other names. Awati regarded them as the appendages of the ninth segment, and I believe that this is the correct interpretation of their true nature, and they would then be homologous with the coxites of the ninth segment (claspers, forceps, etc.) of mosquitoes. It is possible then, if this view be accepted, that the small hairy sclerite on the outer side represents the basal, and the larger sclerite the distal, segment of the coxite. In *Musca* these appendages are obviously very efficient clasping organs. They are well provided with hairs on the inner convex surface and also with what appear to be sense organs; close to the articulation with the lateral process of the ninth tergo-sternum, there are always several long hairs. Although there are slight differences in the structure of these coxites in the different species, the differences are so small and difficult to interpret, unless each is oriented in exactly the same manner (a very difficult matter), that it is not possible to use these characters; they are of more use as generic characters.

SUMMARY OF THE SALIENT CHARACTERS OF THE MALE TERMINALIA IN *MUSCA*

As far as is known at present, no rotation of the terminalia takes place as in the male mosquito and other lower Diptera. The eighth segment has entirely disappeared. The ninth segment is represented by the fused tergum and sternum, and does not form a continuous ring enclosing the appendages, but is a semi-circular plate invaginated into the genital sinus. It bears the phallosome and parameres, and articulates at the sides with its coxites. The phallosome is a simple tube exhibiting no structural change throughout the known species of the genus; it bears no chitinous or other ornamentation, and is provided with no gadgets. This fact confirms the conclusion that the genus is a markedly homogeneous one, consisting of very closely allied species. The posterior part of the paramere exhibits a remarkable development from a simple, small, flat, button-shaped plate in the lowest forms to a long, bent upstanding process in the highest; the anterior part merely becomes larger; the latter always bears bristles, the former minute sense

spines. Correlated with the evolution of the paramere, the posterior process of the phallosome (fig. 4, c) develops from a simple, long, bent, unexpanded rod into a large, broad, hood-shaped, commonly Y-forked process in the highest forms. The appendages or coxites of the ninth segment are remarkably uniform in structure only exhibiting minor differences. It is most important here to note their position and exact relation to the ninth segment and the anal cerci, for then and only then is it possible to trace them in the higher forms of the Muscinae, and in the Anthomyinae, Calliphorinae, etc., in which they often become highly modified and displaced. The anal cerci are triangular plates united together rather loosely by a wedge of chitin, and exhibit a strikingly uniform structure throughout the genus; together they simulate the wings of a butterfly. The two sides of the free (anterior) border, from being rounded, tend, especially the inner, to become produced into nipple-shaped processes, and these reach their highest development in the species of the *lusoria* group.

THE NATURAL GROUPING OF THE SPECIES

I have now made an exhaustive study of the male terminalia of forty-one out of the forty-four species of *Musca* known to me at present, and in all but a few have examined more than one specimen, and in many from six to fifty. As a result I have been able, (1), to establish their relationships to each other, and in particular to discover the true affinities of some of the apparently specialized forms; (2), to rectify a few erroneous determinations previously arrived at by me; and (3), to fix the true identity of the species so that any one can determine them for himself with certainty.

The species fall naturally into three groups, which I propose naming the *domestica*, *sorbens* and *lusoria* groups, using the specific names of three of the oldest species in the groups. The groups are based primarily on the structure of the posterior part of the paramere, and secondarily on the characters of the posterior process of the phallosome, anal cerci and the lateral processes of the fifth sternum. The key to the evolution of the species in the genus lies in the recognition of the development of the posterior part of the paramere

from a small, flat plate into a long process (fig. 9). The *domestica* group contains the simplest forms, the *lusoria* group the most advanced, while the species of the *sorbens* group occupy an intermediate position. The smallest species belong to the *sorbens* group and the largest to the *lusoria*.

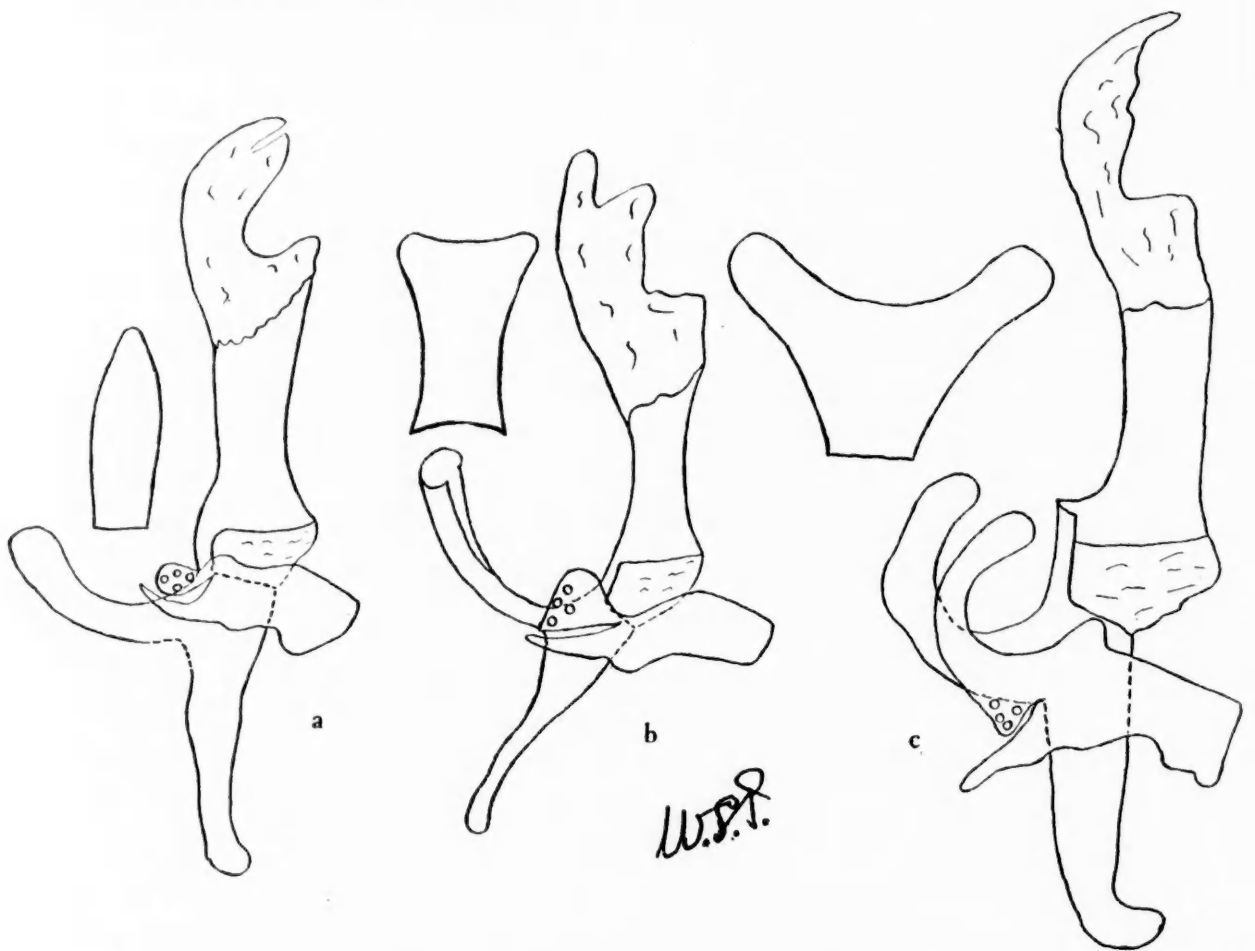


FIG. 9. *a.*—*Domestica* Group. Phallosome and paramere of *M. domestica*—note the small, almost flat, posterior part of paramere, and the unexpanded posterior process; *b.*—*Sorbens* Group. Phallosome and paramere of *Musca sorbens*—note how the posterior part of paramere has now grown into a sugar-loaf like projection, and the posterior process has become expanded, but not Y-forked. *c.*—*Lusoria* Group. Phallosome and paramere of *M. lusoria*—note how the anterior part of paramere has become enlarged, and has a long, backward process where it becomes continuous with the posterior part, which is now a long, upstanding bent process expanded at the end; the posterior process of phallosome is now widely expanded, and deeply Y-forked. Note how the phallosome is remarkably similar in the three groups.

I. DOMESTICA GROUP. TERMINALIC CHARACTERS. PARAMERE. Simple. Anterior part relatively narrow, rounded anteriorly, sloping downwards and backwards into two processes, the outer the longer bending round towards the inner, the two enclosing the posterior part; bearing on the outer surface usually one long and several smaller bristles, the number of these varying in the different

species. Posterior portion consisting of a small, flat, button-like plate, hardly or slightly raised with four, sometimes more, sense spines with round clear bases. POSTERIOR PROCESS OF PHALLOSOME. Either short or long, and practically of uniform width throughout (in two species slightly expanded), even narrowing, and in one

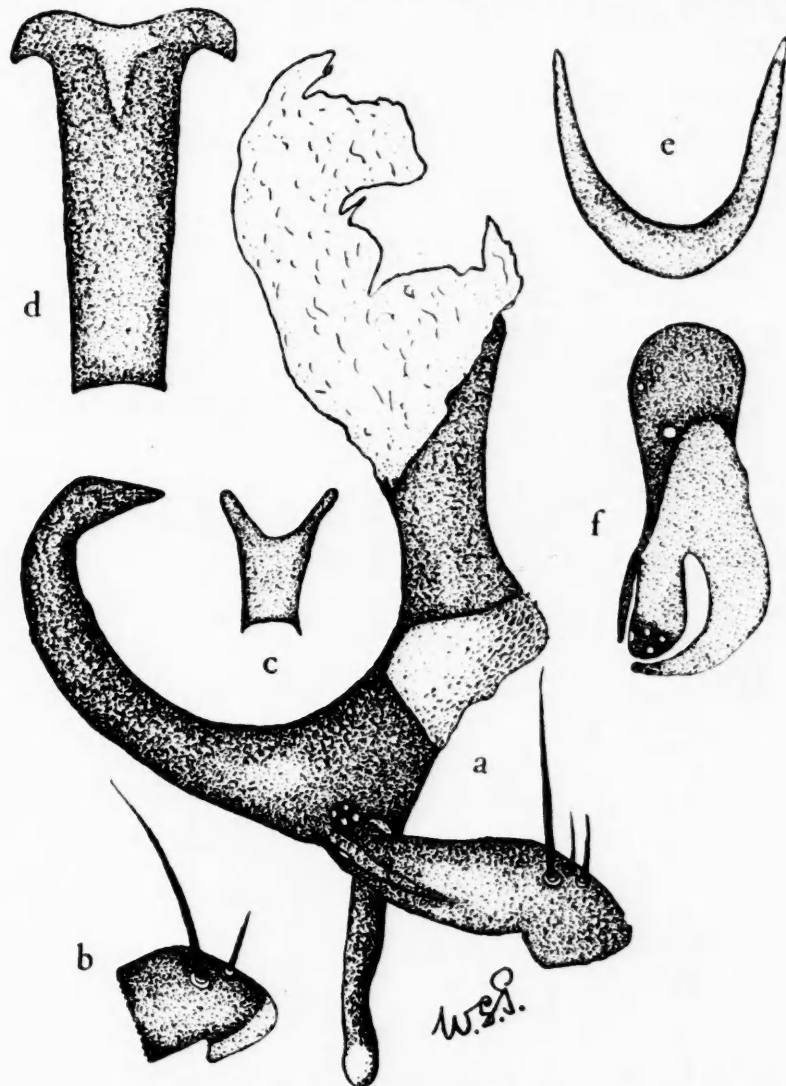


FIG. 10. *a.*—Phallosome and paramere of *M. planiceps*—note the short chitinous part of phallosome, the simple paramere, especially the posterior part, which is almost flat, the very characteristic long, bent, unexpanded, but Y-forked posterior process; *b.*—anterior part of paramere from another specimen, showing only two bristles; *c.*—posterior process showing the forking from front; *d.*—posterior process seen from back; *e.*—forking of posterior process from another angle; *f.*—paramere from above, to show its simple structure, especially the posterior part which is not enlarged.

species very long and forked at the end (*planiceps*). ANAL CERCUS. Outer and inner ends of free margin (anterior) rounded, the latter not tending to form nipple-like processes to any marked extent. LATERAL PROCESS OF FIFTH STERNUM. Long, strongly developed,

or very long and curved, and either well serrated or practically smooth. PHALLOSOME. Chitinous part wide and long—as long or longer than the membranous part.

The following species known to me at present belong to this group:—*domestica*, *vicina*, *nebulo*, *planiceps*, *hilli*, *terrae reginae*, a new Ethiopian species, *vetustissima* and *yerburyi*. *M. domestica*,

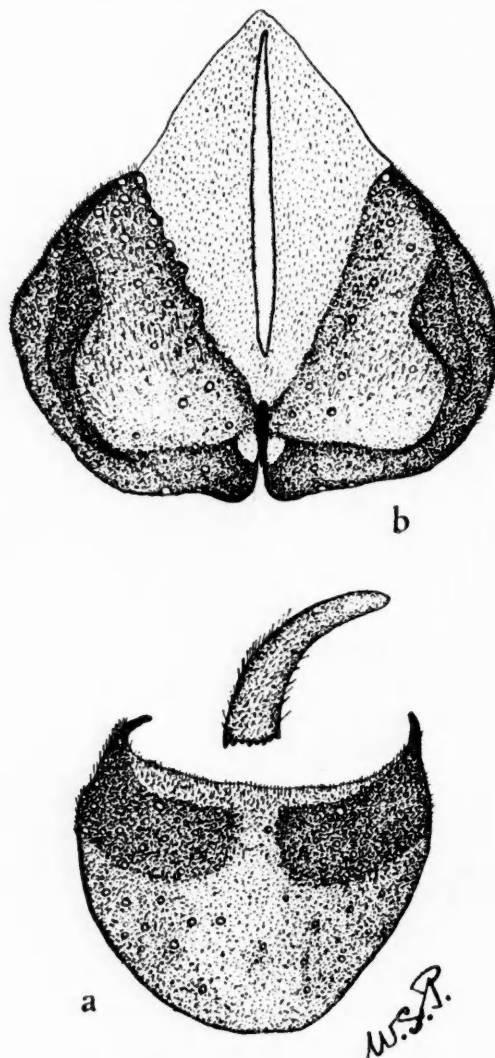


FIG. 11. a.—Fifth sternum of *M. planiceps*—note the rather long, almost smooth posterior process; b.—anal cerci of same.

vicina, *nebulo*, *hilli*, *terrae reginae*, *vetustissima* and *yerburyi* are house, bazaar and camp frequenting species, and are some of man's most dangerous insect pests. *M. domestica* is the European house fly; *vicina* is its tropical ally; *nebulo* is common in India; *yerburyi* is only found in India; *hilli* and *terrae reginae* are only found in Australia. *M. vetustissima* is found in the Australian, Oriental and

Ethiopian regions ; it is a common camp and bush pest. *Musca yerburyi* and *M. vetustissima* are both most advanced species and come very near those of the next group, and it is difficult to be quite sure whether to place them at the end of the *domestica* or at the beginning of the *sorbens* group ; I have decided to place them in the former position.

M. planiceps is of peculiar interest as it is not only a very efficient blood-sucker, but is also larviparous in habit, depositing one larva at a time at the commencement of the third stage ; it feeds mainly on the blood of bovines and the larvae are deposited in fresh cow dung. As far as its reproductive system is concerned it comes very near the species of *Glossina* which, it will be remembered, deposit one larva at a time at the end of the third stage. Although apparently a very specialised species, in being both a blood-sucker and with an advanced larviparous habit, it is in reality one of the simplest forms and is closely related to *domestica* and its allies. The true affinities of this puzzling species could not possibly have been revealed but through a study of its terminalia. The female reproductive system is illustrated in Part I of my book, '*Insects, Ticks, Mites, etc.*' (1929). I have already cleared up the synonymy of this species and examined material from almost throughout the entire range of its distribution, which is the Oriental region. Malloch has apparently re-described it from Formosa under the name *formosana*. I have bred it in large numbers in South India, and have observed and studied it in the field. It is never very plentiful in any locality. The phallosome and paramere are illustrated in fig. 10, *a*. Note the rather short chitinous part of the phallosome, the long bent, very characteristic posterior process, which is of uniform width throughout, and is forked at the extremity, the appearance of the fork varying with the angle at which it is viewed (*c, d, e*). Note also the simple paramere (*a, f*), and especially the posterior part which is quite flat and not raised at all. The fifth sternum and anal cerci are illustrated in fig. 11 ; note the rounded free margins of the latter and the long, almost smooth posterior process of the former. These terminalic characters leave no doubt as to the true identity of this species. I hope to illustrate its proboscis and the characters of the larva in my revision of the Oriental species.

2. SORBENS GROUP. TERMINALIC CHARACTERS. PARAMERE. Still simple. Anterior portion narrow and rounded in front, sloping downwards and backwards, ending in similar processes as in the *domestica* group and enclosing the posterior part; bearing one long and several smaller bristles, the number varying in the different

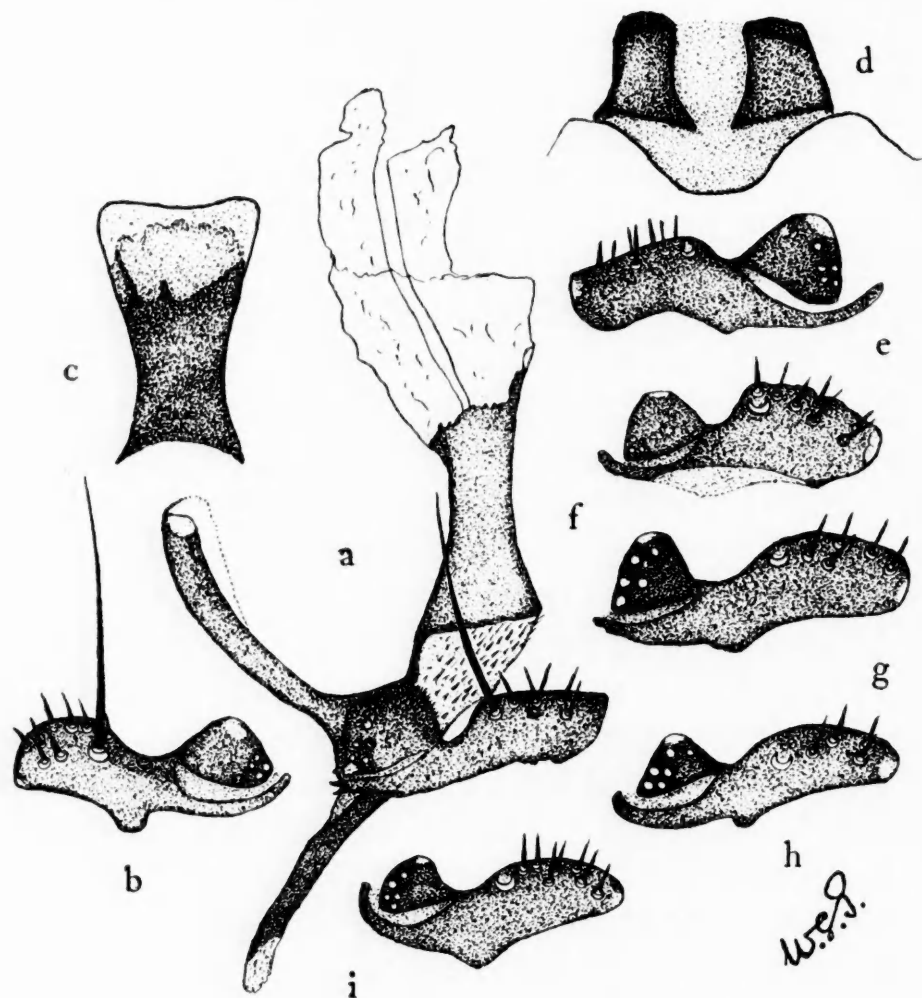


FIG. 12. *a*.—Phallosome and paramere of *M. sorbens* (Sierra Leone)—note the narrow, somewhat waisted, chitinous part of body of phallosome, the broad, mound-shaped, haired posterior portion of paramere, the rather short, expanded posterior process; *b*.—paramere of specimen from India—note the rather low sugar-loaf posterior part; *c*.—front view of posterior process, showing expanded end; *d*.—front view of anterior part of parameres (India); *e*.—paramere of specimen from Sierra Leone, with light 1st and 2nd terga; *f*.—paramere of typical form from Sierra Leone; *g*.—paramere of specimen from Sierra Leone, with dark 1st and 2nd terga; *h*.—paramere of specimen from Mesopotamia; *i*.—paramere of another of same.

species. Posterior part more developed than in the *domestica* group, raised to form a cone-shaped or sugar-loaf process of varying length and width in the different species. The free end is often lightly chitinised, and its edges may have a frayed appearance, or may even be armed with fine hairs. At one side (usually dorsally as it lies in

its normal position), it has four to six sense spines with round, clear bases (in cleared specimens). POSTERIOR PROCESS OF PHALLOSOME. Long and expanded distally, hood-like, tending in some species to fork. ANAL CERCUS. Anterior or free border rounded, the inner end tending to form nipple-like processes; in the majority, however, this is not marked, and there may even be little or no evidence of this change. LATERAL PROCESS OF FIFTH STERNUM. Well developed and relatively long, slightly expanded at end and usually well serrated. PHALLOSOME. Relatively small, chitinous part of body not so stout as in the *domestica* group, inclined to be slender and waist-like in some species, and about equal in length to the membranous part. The phallosome and paramere of *M. sorbens*, a typical member of the group, is illustrated in fig. 12, and anal cerci and fifth sternum in fig. 13.

The following eleven species are known to me at present:—*sorbens*, *ventrosa*, *tempestatum*, *fasciata*, *albina*, *conducens*, *lucidula*, *crassirostris*, *vitripennis*, *interrupta* and *tempestiva*. *Musca sorbens*, *tempestiva*, *fasciata* and probably *tempestatum*, are camp-frequenting species, the first two being particularly objectionable pests, commonly swarming on to the hands and face, attracted by perspiration; they are also found on animals. The remaining species are mainly found on and about animals, and are haematophagous in habit; they are common about cow dung. *M. albina* is found on the edge of deserts and sandy tracts. Most of the species are among the smallest members of the genus, and in the majority the thorax is marked with only two dark stripes; *lucidula* and the male *albina* are metallic. As far as known at present, there is no larviparous species in this group.

M. conducens is of special interest as it exhibits an early phase in the development of the blood-drawing proboscis in this genus. Although it can scratch off a scab, it is unable to tear through the skin and draw blood, the prestomal teeth and the structures concerned in their movement not being sufficiently developed. It is, therefore, still a haematophagous species, and depends for its food on other blood-sucking flies. *Musca crassirostris* is the well-known blood-sucking species, and is a pest of the first order to bovines in India. The late Major Cragg (1912) described its proboscis fully, and showed conclusively that it is identical in structure to that of

Musca domestica; its early stages have been also described by Patton and Cragg (1913). The female lays a relatively large egg which hatches rapidly, suggesting a step towards the larviparous habit. There are two other anomolous species in this group, viz., *M. albina*, in which the sternopleural bristles are absent, and

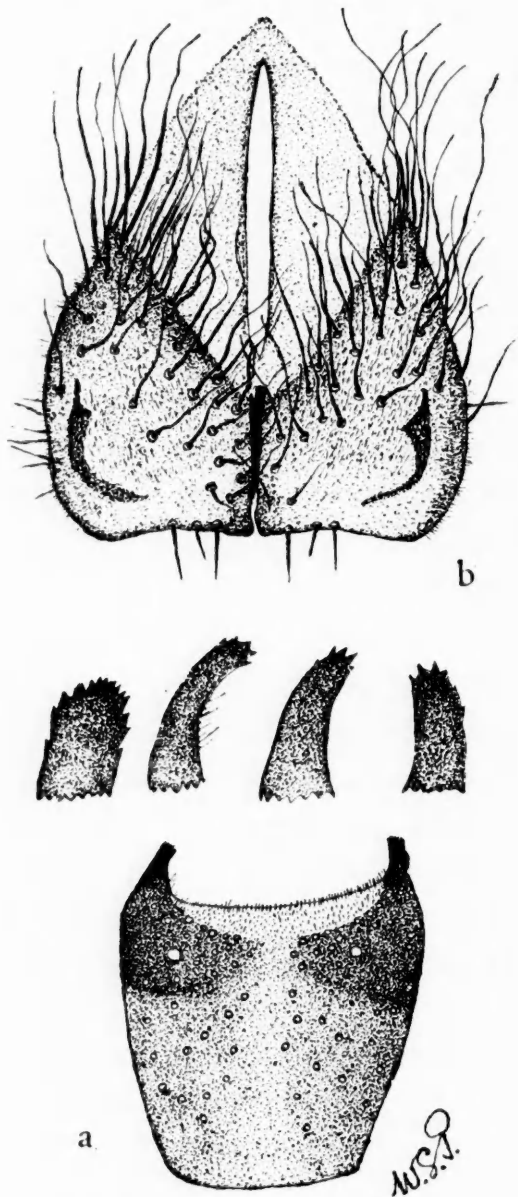


FIG. 13. *a*.—Fifth sternum of *M. sorbens* from India—note posterior processes enlarged, showing various shapes and degrees of serration; *b*.—anal cerci of same.

M. lucidula, in which R_5 is closed by the junction of R_{4+5} and M_{1+2} . In all the other species of the genus the sternopleural bristles are always three in number, arranged 1:2, and cell R_5 is always narrowly open. The male *lucidula*, as pointed out by

Bezzi (1932), is strikingly like the male *albina* in general appearance, but it has the usual three sternopleural bristles; the terminalia of the two species are also similar, clearly showing that they are very closely related (figs. 16, 20). *Musca tempestatum* is peculiar in possessing large, long labella with poorly developed prestomal teeth.

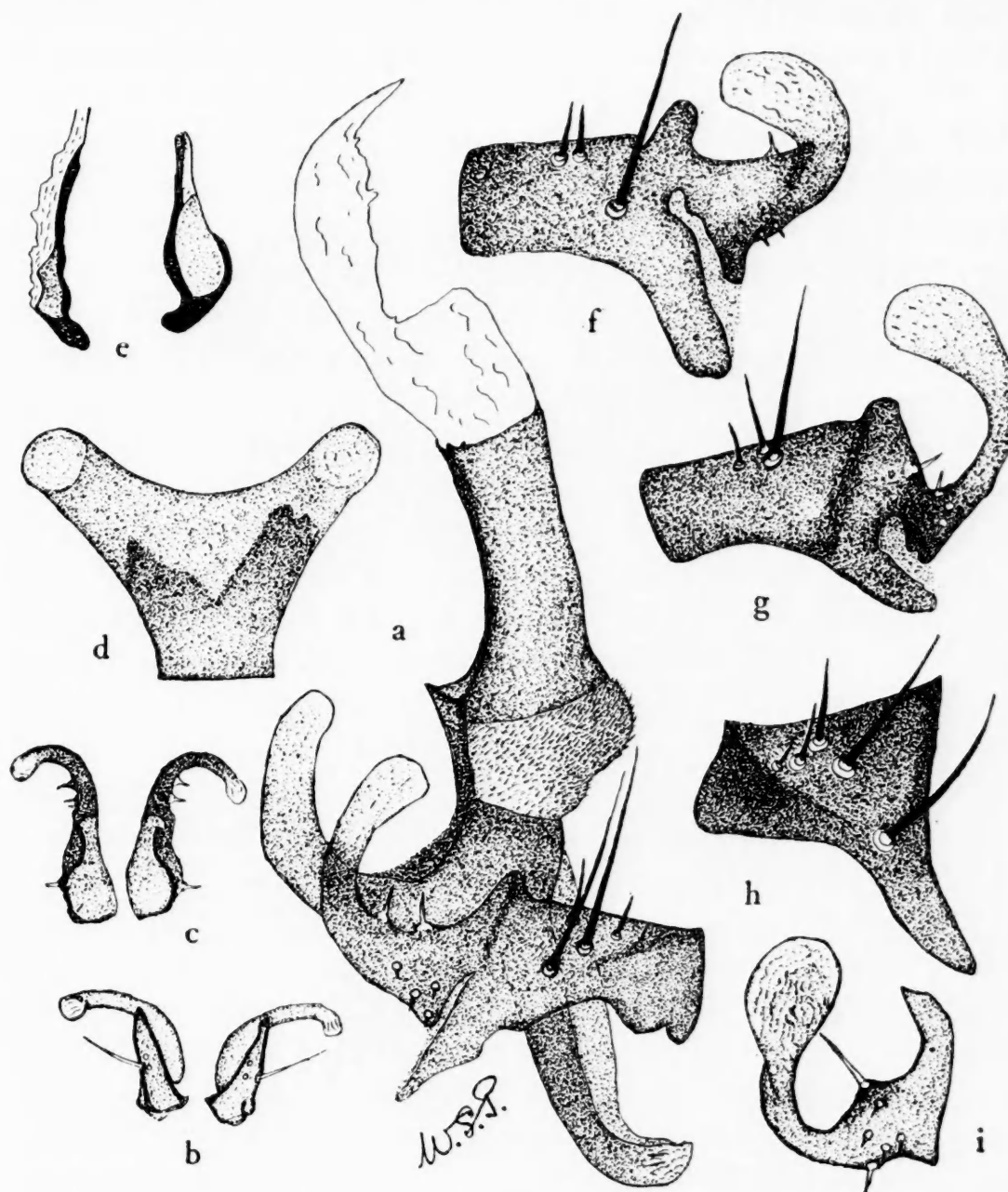


FIG. 14. *a.*—Phallosome and paramere of *M. lusoria*—note the long, stout, chitinous body of phallosome, large paramere, especially the anterior part with stout process and from 3-5 bristles; the posterior part is a long, bent process expanded at end, and has commonly two rather long spines on concave portion, and smaller ones at base; *b.*—front view of parameres—note the long posterior part; *c.*—another front view of same; *d.*—front view of posterior process showing expanded and Y-forked end; *e.*—different views of sclerite of spermatic pump; *f.*—paramere dissected off to show structure; *g.*—another of same; *h.*—anterior end of a paramere to show abnormal bristling; *i.*—posterior part of paramere to show abnormal sense spines—note the dilated end.

3. LUSORIA GROUP. TERMINALIC CHARACTERS. PARAMERE. More elaborate and tending, though still united, to become divided into two distinct parts. Anterior portion enlarged, deeper than in either of the other groups, and extending down on the outer side in a wide process; bearing one or more long bristles and several smaller ones, the latter varying in number and size in the different species and in the two parameres of the same species. Posterior portion elongated to form a longer process than in the *sorbens* group, or a very long, thick, outwardly bent, stout, rod-like process usually expanded at the distal end; bearing four to six or more small sensory spines varying in their position but usually located at the base, but sometimes on the shaft. POSTERIOR PROCESS OF PHALLOSOME. Strongly developed, broadly expanded, hood-like, and nearly always Y-forked, the forks either long or short; in two species (*gabonensis*, *fletcheri*) widely expanded without forking. ANAL CERCI. Large, markedly convex, inner end of anterior (free) border ending in either very long, stout, nipple-like processes (*lusoria*, *spinohumera*), or in relatively smaller, shorter nipples (*inferior*, *gibsoni*, *autumnalis*, etc.); the outer margin in the majority simply rounded, but may form broad nipple-like processes (*lusoria*, *spinohumera*). LATERAL PROCESS OF FIFTH STERNUM. Either moderately long, broad and markedly serrated, or hardly projecting from the anterior margin of the sternum and well serrated (*larvipara*, *bezzii*, etc.). PHALLOSOME. Chitinous part well developed, broad, stout, and either about equal in length to the membranous part, or short to very short, and only about half as long as the membranous part.

The following twenty-three species known to me at present belong to this group; I have not yet had an opportunity of examining the terminalia of the last three, and only provisionally place them here at the end; it is more than probable that *M. lucens* belongs to the *sorbens* group:—*senior-whitei*, *xanthomelas*, *craggi*, *autumnalis*, *hervei*, *gibsoni*, *pattoni*, *gabonensis*, *fletcheri*, *mesopotamiensis*, *villeneuvei*, *illingworthi*, *bezzii*, *fergusoni*, *convexifrons*, *bakeri*, *larvipara*, *natalensis*, *inferior*, *lusoria*, *lucens*, *alpessa* and *dasyops*. This group not only contains the largest number of species, but also the largest and most specialised individual members of the genus; it also contains some small species, viz., *craggi* and *villeneuvei*. They are widely distributed, and representatives are to be found in every part

of the world except North and South America. They are only found out-of-doors, are generally very abundant about animals, and are some of the most serious pests of farm animals. The group contains some interesting anomolous species. *Musca inferior* is the only known species which has a group of small hairs on the upper

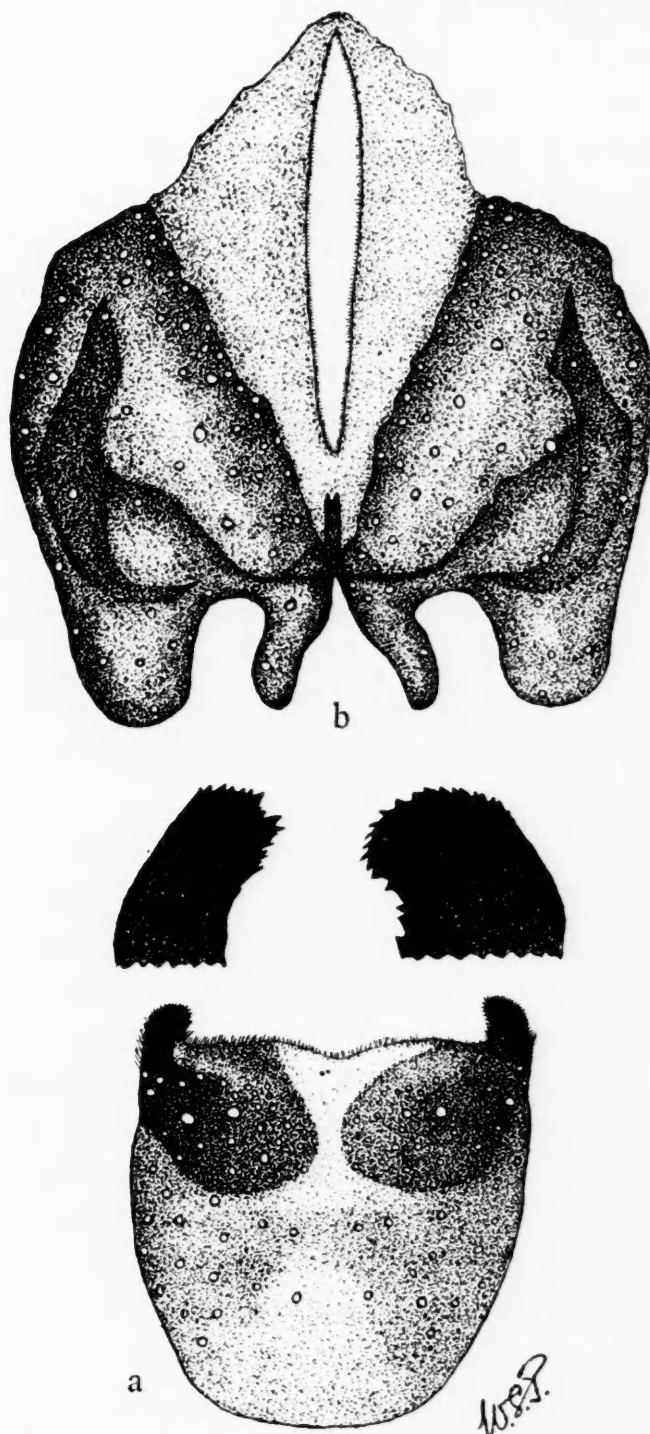


FIG. 15. *a.*—Fifth sternum of *M. lusoria* and posterior processes enlarged to show variation in structure—note the small size (relatively to others) of the sternum; *b.*—anal cerci—note the long, nipple-shaped processes.

surface of the squama, and the vertex of the male is relatively wide, as in the male *domestica*; indeed, several observers have drawn attention to the similarity existing between the male *domestica* and the male *inferior*. This species is an efficient blood-sucker. I do not know whether it is larviparous or not. There are two other blood-sucking species in the group, *M. senior-whitei* and *M. fletcheri*; the latter bears a superficial resemblance to *inferior*, and the male vertex is also wide. *Musca mesopotamiensis* is of peculiar interest for like *M. conducens* in the *sorbens* group, it has quite well-developed prestomal teeth and can scratch off a scab; it is a relatively large species whereas *conducens* is small. Many of the species of this group are larviparous in habit, such as *M. lusoria*, *bezzii*, *larvipara*, *fergusoni* and probably others; the larvae, as far as is known, are always deposited at the commencement of the second stage. The reproductive organs of *M. bezzii* are illustrated in Part I of my book (1929). Many of the oviparous species deposit pediceled eggs, and both the oviparous and larviparous species have white or dirty white puparia.

In most of the species of this group vein R_{4+5} has, on the ventral side, a row of small bristles well spaced out and extending almost to the tip. But they are also present on R_{4+5} in *M. planiceps*, which belongs to group 1. It is clear, therefore, that they are of no value as group characters.

A careful comparison of the male terminalia of *M. prashadi* with those of *M. autumnalis* has resulted in my sinking this name as a synonym of the common European haematophagous species. I have been able to reinstate the name *fergusoni* for the common larviparous Australian species. It will be remembered that I sank this name as a synonym of *convexifrons*, after comparing the type male and only known specimen of Thomson's Chinese species with males of *fergusoni*. When in China I was fortunate in collecting Thomson's species, and after comparing its terminalia with those of *fergusoni*, and also comparing the females, which I had no opportunity of doing before, I have come to the conclusion that both are distinct species. The phallosome, paramere, anal cerci and fifth sternum of *M. lusoria*, a typical member of the group, are illustrated in figs. 14, 15.

A little further consideration of the arrangement of the species

into the three groups on terminalic characters will bring out very significantly the real importance and meaning of these characters in understanding the true relationships of the species to each other, and their evolution within the genus. As a result of this comparative study, we have convincing evidence of the gradual development of a structure, the paramere, from an obviously simple condition to a more complex form (fig. 9). If these developmental changes were found to arrange themselves strictly into three water-tight compartments, that is to say, if the species of each group all exhibited the same amount of development of the paramere, and were thus sharply separated from the species of the next group, one would then be at least suspicious that this structure did not give a true clue to the evolution of the species in the genus. But here we have some species in each group clearly exhibiting a development towards the next phase, so that it becomes very difficult to decide whether they should be placed at the end of one group, or at the beginning of the next; in other words, the species of the groups tend to merge into each other, a condition always true to nature. *Musca yerburyi* and *M. vetustissima* could as well be placed at the beginning of the second group as at the end of the first. In the same way, *M. senior-whitei* might just as well have been placed at the end of the second group as at the beginning of the third. It is also very significant to note that it is that part of the paramere which carries the sense spines (at least they are believed to be such) which has undergone this remarkable development. It is interesting to note too that the anal cercus, the posterior process of the phallosome, and the posterior process of the fifth sternum are all to a certain extent correlated structurally with this development of the paramere. Although all these parts exhibit evidences of gradual development from a simple condition to a more complex, the phallosome has remained throughout practically unchanged in its original simple form, even in the most advanced species, a further, and in my opinion, conclusive proof that the genus consists of a homogeneous concept, which cannot possibly be split into isolated genera but only arranged into related groups.

The fact that the five known blood-sucking species, *senior-whitei*, *fletcheri*, *planiceps*, *inferior* and *crassirostris* are distributed among the three groups, and do not all fall into one, is further proof of the real significance of the terminalic characters in revealing the

true status of the species. It will be remembered that these species fall into the following groups :—

- | | |
|-----------------------------|---------------------------|
| (1) <i>domestica</i> group. | <i>Musca planiceps</i> . |
| (2) <i>sorbens</i> ,, | ,, <i>crassirostris</i> . |
| (3) <i>lusoria</i> ,, | ,, <i>senior-whitei</i> . |
| | ,, <i>fletcheri</i> . |
| | ,, <i>inferior</i> . |

Of these five species, *crassirostris* has the most highly developed blood-drawing proboscis, and yet it will be noted that it belongs to the intermediate group, its terminalia not exhibiting the highest development. *M. inferior* has a less highly developed blood-drawing proboscis but has more highly developed terminalia, and is obviously a specialised species in this respect, and belongs to the *lusoria* group. *Musca planiceps* has a still less highly developed, though most efficient, blood-drawing proboscis, and is, on its terminalic characters, closely allied to the simplest species. The two remaining species are probably as efficient blood-suckers judging from the structure of the proboscis, and they both belong to the third group, *M. fletcheri* being the more specialised of the two and closely related to an African species, *M. gabonensis* (*scatophaga*), which, from an examination of its proboscis, is not a blood-sucker.

Further, there is in each of the second and third groups a single species which exhibits the early phase in the evolution of the blood-drawing proboscis, viz., *M. conducens* in group 2, and *M. mesopotamiensis* in group 3. All these facts clearly demonstrate that in this genus the blood-drawing proboscis has been independently evolved in each group along the same lines, and in two, more than once. Further, this grouping of the species based on terminalic characters also clearly demonstrates the truth that the larviparous habit has also evolved independently in at least two of the groups. And it is interesting to note that its most advanced state, as exemplified in *M. planiceps*, in which a larva is deposited one at a time in fresh cow dung in the early third stage (after being retained *in utero* during two moults) can be quite consistent with simple terminalic characters. This fact was demonstrated by Newstead (1911), in his study of the terminalia of *Glossina*. These important flies, though exhibiting a very advanced stage in the larviparous habit, still possess extremely primitive terminalia, particularly

noticeable in the paramere (inferior clasper) and the anal cerci (superior claspers) in the *fusca* group. I have been fortunate in being able to study the male terminalia of all the known species of *Glossina*, and will, in another paper on the revision of the genera of the Muscinae, discuss the relationships of the species of *Glossina* to each other and to related genera.

PREVIOUS ATTEMPTS AT GROUPING THE SPECIES OF *MUSCA*

Having now explained how the species of *Musca* arrange themselves naturally into three groups on the characters of the male terminalia, it is necessary to review shortly previous attempts to classify the species on characters other than the male terminalia. Robineau-Desvoidy (1832) erected the genera *Biomyia* and *Placomyia* (*Byomyia*, *Plaxemya*) for the species *tempestiva* and *vitripennis* respectively, the former possessing bare, the latter hairy, eyes. These two genera were retained by most of the older authors. Austen (1909) erected the genus *Philaematomyia* for *crassirostris* (*insignis*), mainly on the supposedly peculiar characters of the proboscis; and Brunetti (1919) erected the genus *Pristirhynchomyia* for *conducens* (*lineata*), also on the characters of the proboscis, the structure of which he unfortunately wrongly interpreted. Bezzi (1911), who was apparently the first to have attempted a grouping of the species, accepted these genera, and gave an elaborate key to the species known to him at the time, and arranged them in seven sections. It is not clear, however, whether Bezzi considered the species in the different sections as being related to each other. As his identifications of the species is now long out of date, no useful purpose would be served by referring to his classification. Townsend (1911, 1915) appears to be the next who has attempted to classify the species. He erected the genus *Eumusca* for *autumnalis* (*corvina*), *Promusca* in place of *Musca* for *domestica*, *Viviparomusca* for *bezzii*, and *Awatia* for *planiceps*. Were it not for the fact that most of these genera have been retained by Malloch, I would have passed over this classification without referring to it. Even a superficial examination of a few species belonging to these genera of Townsend reveals the

fact that the characters given as defining them are not even of specific value. The species of *Promusca*, for instance, are said to be distinguished by the wide frontalia of the female and the wide vertex of the male. The female is said to have from sixty to eighty ovarioles, a pair of copulatory vesicles, and it lays a small, unpediceled, unmodified, unincubated egg, and all the eggs are deposited at one act of oviposition, and the puparium is reddish brown. But the wide female frontalia is to be seen in species of *Eumusca* and *Viviparomusca*, and the males of *inferior* and *fletcheri* have a wide vertex like the male *domestica*. These would be classed by Townsend as atypical members of atypical sub-genera of his genera. The use of characters of the female reproductive organs, the egg, and the colour of the puparium to define genera is not only valueless, but would be of little use to any one attempting to place his species. In this classification, Townsend exhibits his complete ignorance of the species and of the very essentials of systematic Dipterology.

Bezzi (1921) erected the genus *Ptilolepis* for *inferior* on the presence of hairs on the upper surface of the squama, and later (1922) the genus *Lissosterna* for *albina* in which the sternopleural bristles are wanting.

Malloch (1925-1929) has contributed a series of papers on the species of *Musca*, and in the first he gives a key to what he describes as segregates which are based on certain group characters; these are the following:—(1) The presence or absence of propleural hairs. (2) The presence or absence of setulose hairs on the suprasquamal ridge, and (3), the presence or absence of an antero-ventral bristle on the mid tibia. It is necessary to state here that, after examining material determined by this author, and studying his descriptions of species new and old, I find that there is much confusion regarding the identity of many of them, and several have been re-described as new. For instance, he refers to the common Ethiopian species *lusoria* as having two black thoracic stripes, whereas it has four; *M. gibsoni* is said to occur in Australia, but as far as I know, it does not occur outside India; he has re-described *M. xanthomelas* under the name *alba*, and *gabonensis* under the name *scatophaga*. I am very doubtful as to the identity of his Sumatran species, *jacobsoni* and *tibiseta*. The types of these are said to have been deposited in the Zoological Museum at Amsterdam, but Professor de Mejiere tells me

he has failed to find them, so that they must still be in America. I strongly suspect that both have already been described.

Musca formosana appears to be an abnormal specimen of *M. planiceps*, for this species occurs in Formosa; and *M. polita* is probably an abnormal male of *M. albina*. In any case, I propose regarding these species as doubtful until I have had an opportunity of examining the types. Malloch recognises the following genera as distinct and includes in them the species noted.

CLASSIFICATION OF MUSCA ACCORDING TO MALLOCH

Genus *Musca* L (sens. rest.). Species—*domestica*, *sorbens* (*angustifrons*) and *albo-maculata*; this last is a doubtful species.

Genus *Biomyia* R-D. Species—*conducens*, *planiceps*, *ventrosa*, *vetustissima*, *tempestiva*, *tempestatum* and *xanthomelas* (*alba*).

Genus *Placomyia* R-D. Species—*Vitripennis*, *interrupta*, *gibsoni* and *dasyops*.

Genus *Emusca* Malloch (*Eumusca* Townsend). Species—*autumnalis*, *gabonensis* (*scatophaga*) and *craggi*.

Genus *Viviparomusca* Townsend. Species—*larvipara* (*vivipara*), *lusoria*, *pattoni*, *bakeri* and *natalensis*.

Genus *Philaematomyia* Austen. Species—*crassirostris*.

Genus *Ptilolepis* Bezzi. Species—*inferior*.

Genus *Lissosterna* Bezzi. Species—*albina*.

It will be noted that in this classification the old genus *Musca* is split into no less than eight genera on the presence or absence of bristles, hairs, etc. In view of the increasing importance of these flies in both human and veterinary medicine, and as this grouping may be accepted by those who are not in a position to criticise it or judge of its merits, and most important of all, in view of its bearing on the grouping of other species of the higher Diptera on similar characters, it becomes very necessary to examine in detail the real status of these genera in the light of my work on the terminalia.

1. Genus *Lissosterna* Bezzi. *Musca albina* is the only known species without sternopleural bristles, and on the strength of this single character it is placed in an isolated position in a genus by itself. In accepting the validity of this genus, Malloch follows Bezzi. A study of the male terminalia (fig. 16), shows that it belongs to the second group, and in that is closely related to *sorbens*,

vitripennis, *interrupta*, *conducens*, *tempestiva*, *tempestatum*, *ventrosa*, *lucidula* and *crassirostris*; all these species have well developed sternopleural bristles. The conclusion then is that the absence of these bristles in one species has no significance, and its isolation in a genus distinct from its allies gives a totally erroneous idea of its true status. It has other apparently anomalous chaetotactic characters, which also have no phylogenetic meaning. *Musca albina* is, on its male terminalic characters, a typical member of the genus *Musca* (sens. lat.), and should not be placed in a separate genus.

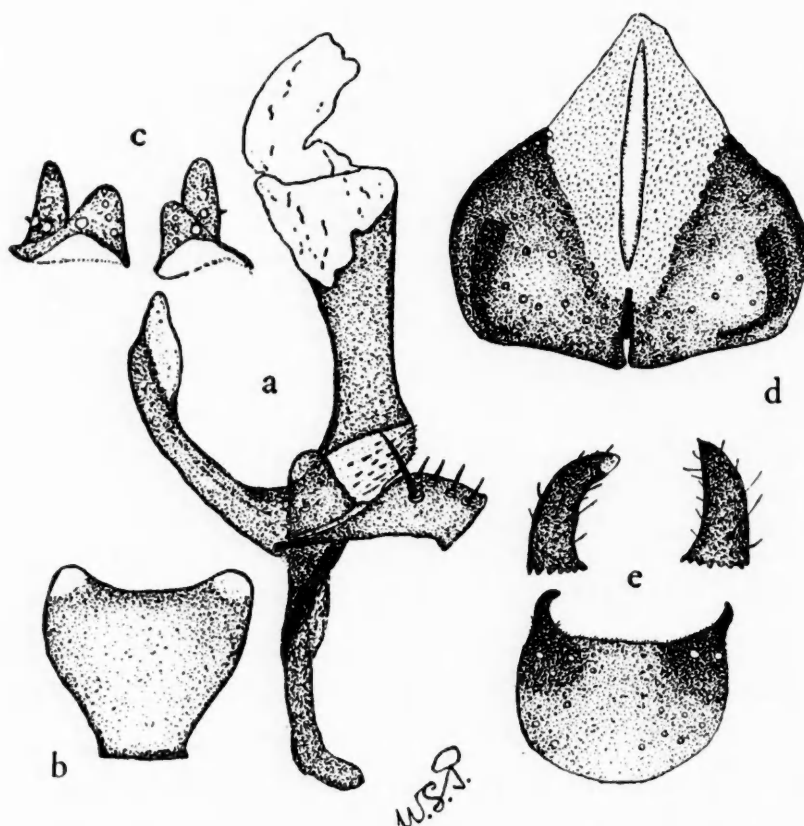


FIG. 16. a.—Phallosome and paramere of *M. albina*—note the large, rounded, sugar-loaf posterior part of paramere, and the narrow, waist-like, chitinous part of body of phallosome; b.—front view of posterior process showing expanded end and suggestion of forking; c.—front view of parameres—note the raised posterior process at sides of both; d.—anal cerci; e.—fifth sternum with posterior process enlarged. All characters clearly proving close relationship with *sorbens* and allies.

2. Genus *Ptilolepis* Bezzi. *Musca inferior* is the only species which possesses hairs on the upper surface of the squama, and on this single character it is placed in a distinct genus, and is isolated from its near allies. It is a blood-sucker, and its proboscis is structurally like that of *crassirostris* and is otherwise typical of the genus; it should, therefore, be placed in the genus *Philaematomyia*. A study

of its male terminalia shows that it belongs to the third group, and in it is closely related to such species as *autumnalis*, *hervei*, *mesopotamiensis*, etc. Much has been made of the presence of hairs on the upper surface of the squama, not only in this species but also in the genera of the Calliphorinae. Bezzi hinted at the possibility that the presence of hairs on the squama in this one species suggests

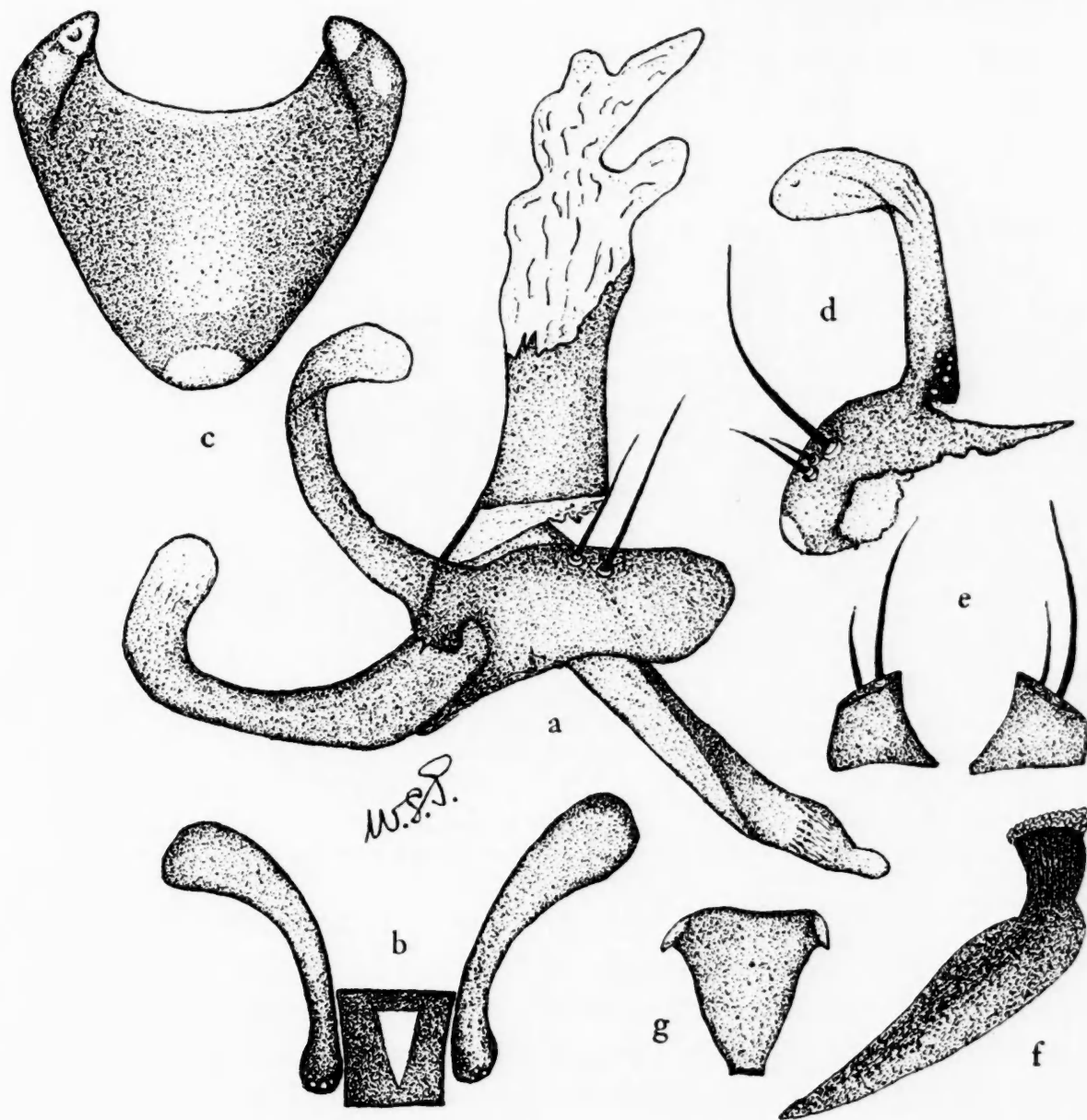


FIG. 17. *a.*—Phallosome and paramere of *M. inferior*—note the short chitinous part of body, the large paramere, especially the long, bent posterior part, and the long posterior process; note the characteristic spines near base of posterior part of paramere; *b.*—front view of posterior part of parameres—note the stout ends; *c.*—front view of posterior process showing expansion and forking; *d.*—paramere dissected off to show structure—note three bristles instead of usual two, also spines at base; *e.*—front view of anterior ends of parameres showing the usual two bristles; *f.*—sperm pump sclerite; *g.*—another front view of posterior process showing expansion.

some Calliphorine relationship. *M. inferior* is in no way related to any Calliphorine. It is further an apparently anomalous species, as the male has a wide vertex like the male of *domestica*, which it rather simulates. On its terminalic characters it is a typical *Musca*.

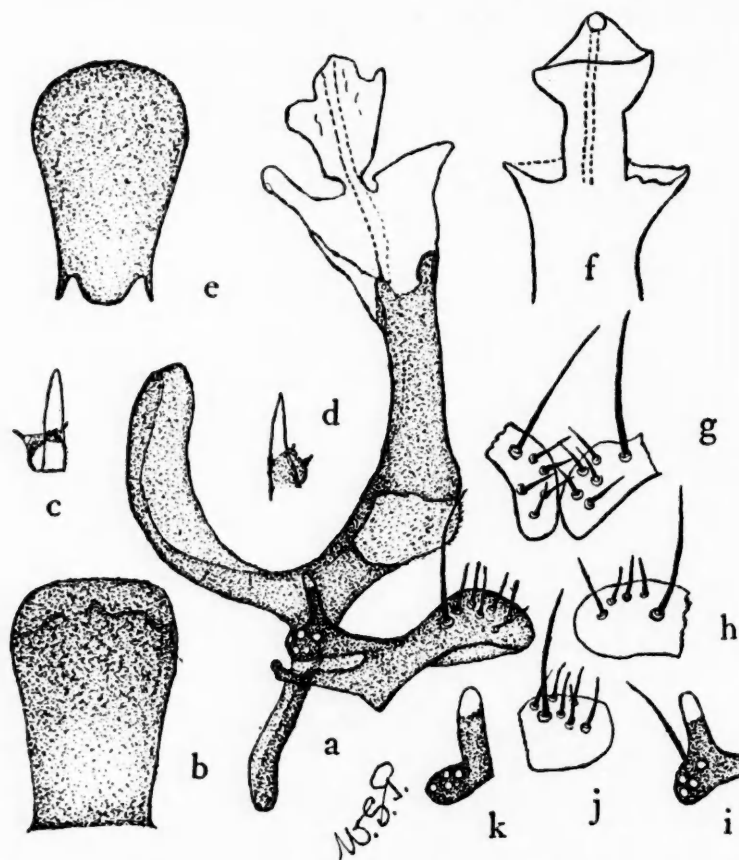


FIG. 18. *a*.—Phallosome and paramere of *M. crassirostris*—note the narrow chitinous part of body, and the rather long posterior part of paramere and the expanded posterior process; *b*.—posterior process showing expanded end; *c*.—posterior part of paramere showing pointed end; *d*.—another of same seen from front; *e*.—posterior process from front showing expanded end; *f*.—membranous part of phallosome showing opening of ejaculatory duct; *g*.—anterior part of paramere showing number and arrangement of bristles; *h*.—side view of anterior part of paramere showing another arrangement of bristles; *i*.—posterior part of paramere showing sense spine replaced by bristle; *j*.—anterior part of paramere showing another arrangement of bristles; *k*.—rather wider specimen of posterior part of paramere.

3. Genus *Philaematomyia* Austen. *Musca crassirostris* was placed by Austen in the genus *Philaematomyia* mainly on the structure of the proboscis. Austen's description would give anyone who did not understand the structure of this proboscis, the idea that it was something quite distinct from that of *Musca domestica*, but, as already noted, it is exactly similar. If the characters of this proboscis be accepted as of generic value, then it would be necessary to place *M. conducens*, *M. mesopotamiensis*, *M. senior-whitei*, *M. fletcheri*, *M. planiceps* and *M. inferior* also in the genus *Philaematomyia*. Malloch retains this genus and gives the following

characters for it :—Mid-tibia with a distinct antero-ventral bristle beyond middle ; proboscis conspicuously thickened, rounded and glossy below ; suprasquamal ridge bare. A study of the male terminalia (fig. 18) shows that it belongs to group 2, and in it is very closely related to *M. lucidula* (fig. 20). The posterior part of the paramere in each is extended into a characteristic attenuated process ; the posterior process of the phallosome is similarly expanded hood-like, but not forked ; the cerci in both have rounded anterior margins, and the posterior process of the fifth sternum is very similar in both. *M. lucidula* is a metallic fly, and is of peculiar interest as it is the only species in which cell R_5 is closed, and it has, therefore, been regarded as distinct from the other species. But there can be no doubt that both these species are related to *sorbens* and the others in group 2. There is clearly, therefore, no justification for the genus *Philaematomyia* for *crassirostris* is a typical *Musca*.

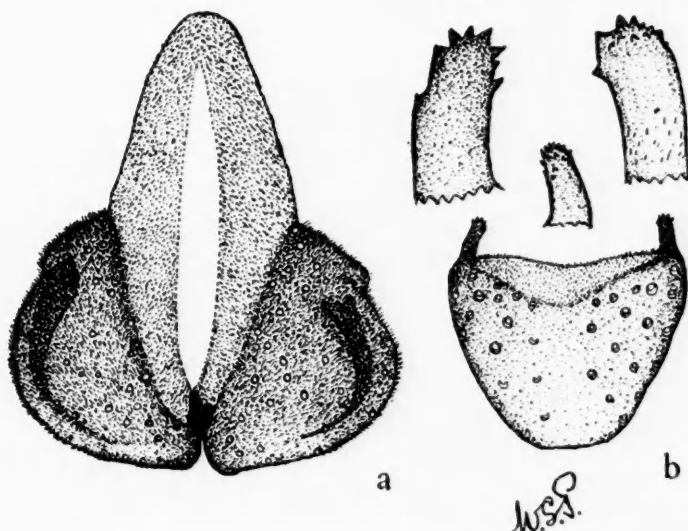


FIG. 19. a.—Anal cerci of *M. crassirostris*—note rounded ends rather like those of *domestica* group ; b.—fifth sternum of same, and posterior process enlarged.

4. Genus *Biomyia* R-D. Malloch places in this genus the species *conducens*, *planiceps*, *ventrosa*, *vetustissima*, *tempestiva*, *tempestatum* and *xanthomelas* (*alba*). According to this author the genus is based on the absence of hairs on the part of the thorax, which he refers to as the suprasquamal ridge at the lower margin of the declivous portion of the mesonotum ; the presence or absence of hairs on this ridge is said to be ' quite an important character in this and related genera '. In the first place, it is necessary to point out that it is exceedingly difficult to examine this particular area, for it is necessary to have the wing bent downwards, and then these

minute hairs can only be seen with the aid of a strong lens and best with a binocular and a good light ; if the fly has hardened with the wings in any other position, it is necessary to remove one wing before the hairs can be seen, and even then a pocket lens is useless. If this is really an important character, the species not possessing these hairs should in other respects be related to each other, and especially on the characters of the male terminalia.

According to the characters of the male terminalia the species of *Biomyia* fall into the following groups :—

Musca conducens belongs to Group 2

„	<i>planiceps</i>	„	„	„	I
„	<i>ventrosa</i>	„	„	„	2
„	<i>vetustissima</i>	„	„	„	I
„	<i>tempestiva</i>	„	„	„	2
„	<i>tempestatum</i>	„	„	„	2
„	<i>xanthomelas</i>	„	„	„	3

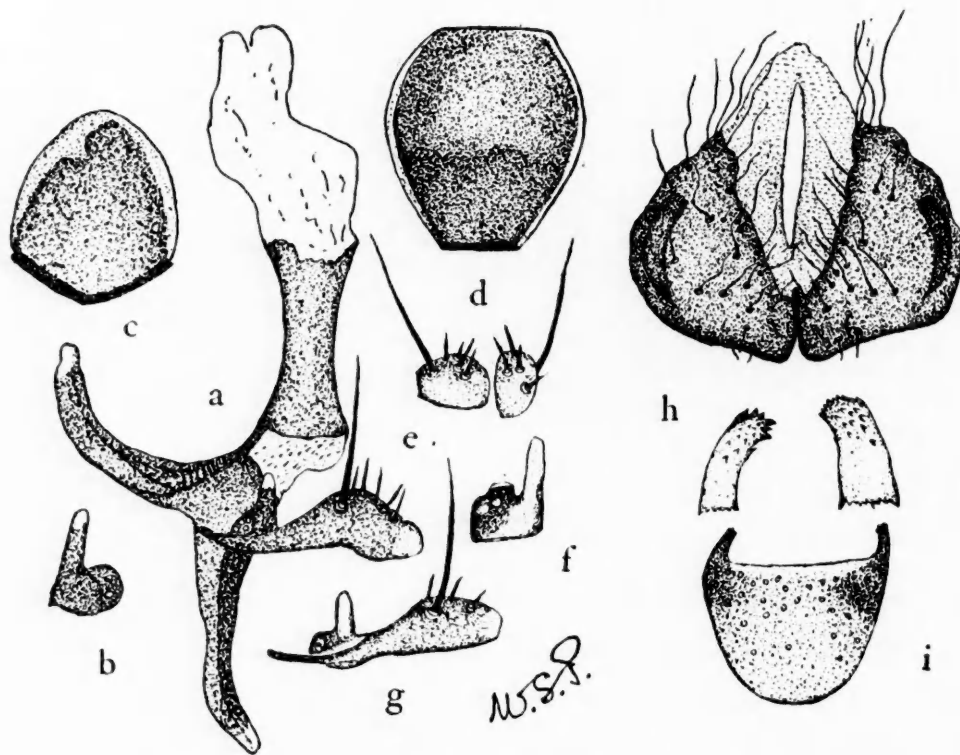


FIG. 20. a.—Phallosome and paramere of *M. lucidula*—note narrow chitinous part of body and long narrow posterior part of paramere, and expanded posterior process of phallosome; b.—another view of posterior process of paramere; c.—expanded posterior process of phallosome from front; d.—another of same; e.—front view of anterior part of paramere showing arrangement of bristles; f.—another view of posterior part of paramere; g.—paramere dissected off to show structure; h.—anal cerci of same; i.—fifth sternum with posterior process enlarged. All these characters demonstrate close affinity with *M. crassirostris*, quite the opposite to affinities based on hairs and bristles.

On terminalic characters, then, it is clear that the species of *Biomyia* belong to each of the groups. *M. planiceps* is distantly related to *conducens*, and still more distantly to *xanthomelas*. This character, therefore, is misleading and its importance has been exaggerated. Malloch has also used it in the genera containing the metallic Muscinae, and here too I have evidence that it is not a generic character.

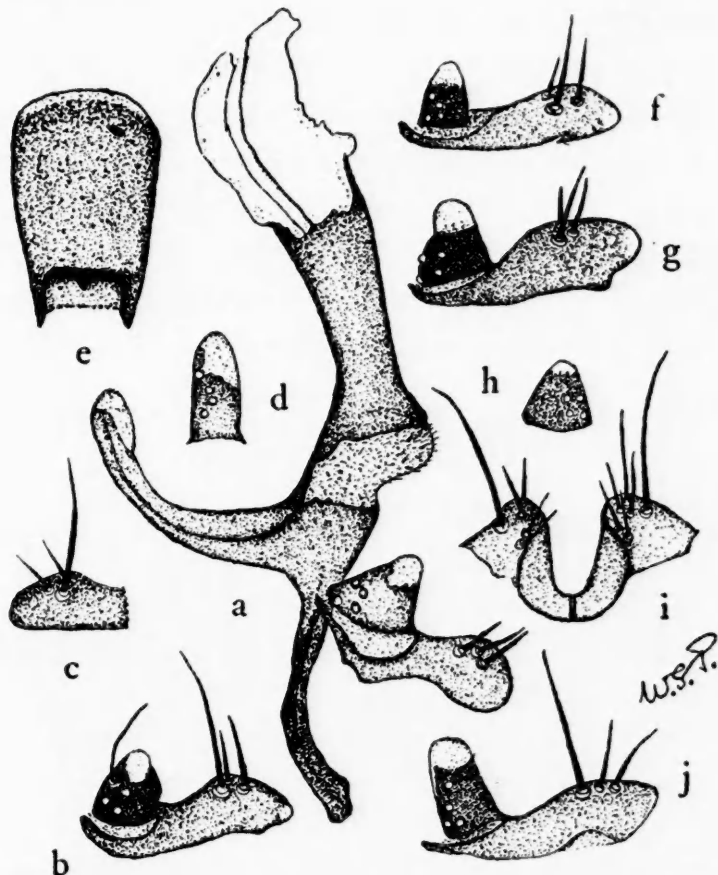


FIG. 21. a.—Phallosome and paramere of *M. conducens* from North India—note sugar-loaf posterior part of paramere, and expanded posterior process; b.—paramere of specimen from Madras—note one of the sense spines has become a long hair; c.—anterior part of paramere from Northern Nigeria; d.—posterior part of paramere of specimen from Madras; e.—front view of posterior process to show expansion of end (India); f.—paramere of specimen from Gold Coast; g.—paramere of specimen from Rabaul, New Britain, Oceania; h.—posterior portion of paramere of specimen from Nigeria; i.—front view of anterior part of parameres of specimen from Madras; j.—paramere of specimen from Zululand—note the rather strongly developed posterior process as in the specimen from Rabaul.

5. Genus *Placomys* R-D. According to Malloch, this genus is a segregate, related to *Biomyia* in that there are 'no setulose hairs at lower anterior extremity of suprasquamal declivity,' but differs in that the eyes are haired in both sexes. In this genus he includes *vitripennis*, *interrupta*, *gibsoni* and *dasyops*. The first two are certainly related: *M. gibsoni* belongs to group 3 and is only

found in India ; I have not yet had an opportunity of examining the male terminalia of *M. dasyops*, but would expect it also to fall in my group 3. Hairiness of the eyes is a thoroughly misleading character, not only in this genus but also in the Anthomyinae ; it is not of generic value. The terminalia of *M. vitripennis* are illustrated in fig. 23.

6. Genus *Musca* L (sens. rest). According to Malloch, all that remains of the old genus *Musca* is characterized by the presence of hairs on the middle of the propleuron, the suprasquamal ridge is bare, and the mid tibia is without a strong antero-ventral bristle. As far as I can gather, Malloch only recognises two species, *domestica* and *sorbens* (*angustifrons*). On the characters of the male terminalia these two species are not related. Here again there is no evidence that the absence of hairs on the suprasquamal ridge has any significance in the grouping of the species.

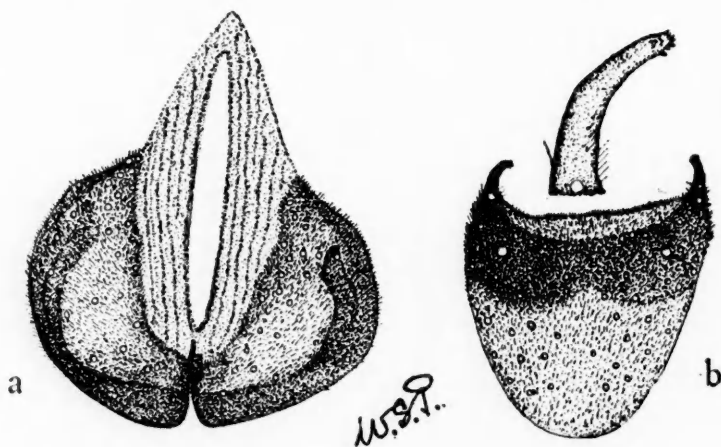


FIG. 22. (a)—Anal cerci of *M. conducens* ; b.—fifth sternum of same—note the long posterior process.

7. Genus *Emusca* Malloch (*Eumusca* Townsend). According to Malloch, the species of *Emusca* can be distinguished by the several [sic] black hairs at the lower anterior extremity of the suprasquamal ridge or declivity. In this genus he places *autumnalis*, *gabonensis* (*scatophaga*) and *craggi*. I have recently had a series of *scatophaga* sent me by Mr. Munro, among which were several determined by Curran as being Malloch's species, and Malloch's description agrees exactly with them. *M. scatophaga* is, however, identical with *M. gabonensis* Macq., the type of which is in Bigot's collection, and which I have examined ; it has a characteristic white puparium. *Musca craggi* is a good species, the male terminalia (fig. 24) being

quite distinct from any known to me at present. I had previously sunk the name as a synonym of *pulla* Bezzi, after carefully examining Bezzi's type and comparing it with my own type and paratypes of *M. craggi*. Malloch now states that *M. pulla* is in reality identical with *M. conducens*; this I cannot agree with and must leave its identity until I can re-examine the type. These three species *autumnalis* (fig. 25), *gabonensis* (fig. 26) and *craggi* (fig. 24) all belong to the third group, and are closely related to all the species which are placed in the genus *Viviparomusca* Townsend, and I therefore see no necessity of isolating them in a separate genus by themselves.

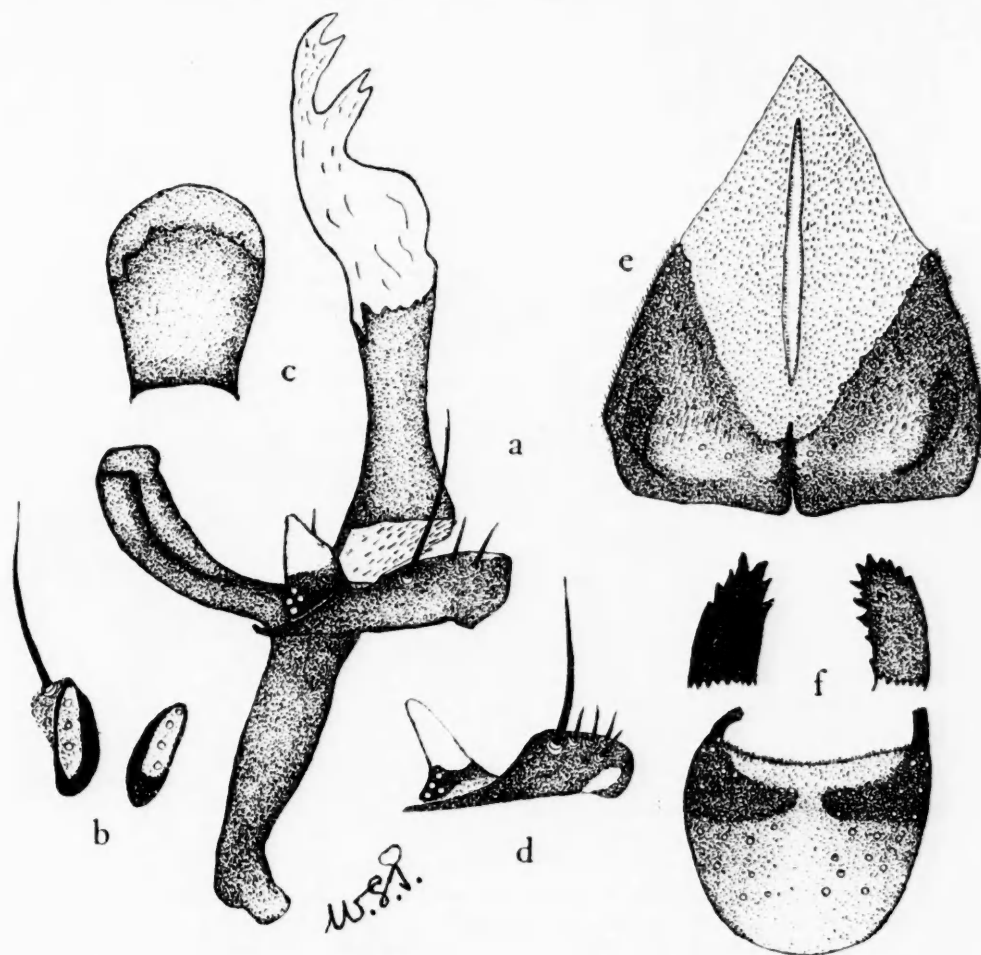


FIG. 23. *a.*—Phallosome and paramere of European form of *M. vitripennis*—note the narrow chitinous body of phallosome, the large conical posterior part of paramere and the expanded posterior process; *b.*—anterior part of parameres from front; *c.*—posterior process showing expanded end; *d.*—paramere of specimen from Khartoum; *e.*—anal cerci; *f.*—fifth sternum and ends of posterior process enlarged.

8. Genus *Viviparomusca* Townsend. In this genus, Malloch places the species *larvipara* (*vivipara*), *lusoria*, *pattoni*, *bezzii*, *bakeri* and *natalensis*, and according to him they are not really distinct

from the species of *Emusca*, as the suprasquamal ridge has setulose hairs in *Viviparomusca*, and in *Emusca* the spurasquamal declivity has 'several black hairs at lower anterior extremity.' All the species listed above, and many more, belong to my third group and are all related, but their relationships are not based on the presence of a few minute hairs but on thoroughly reliable terminalic characters. In all other respects these species are typical members of the genus *Musca*. I, therefore, see no reason for separating them and placing them in a distinct genus.

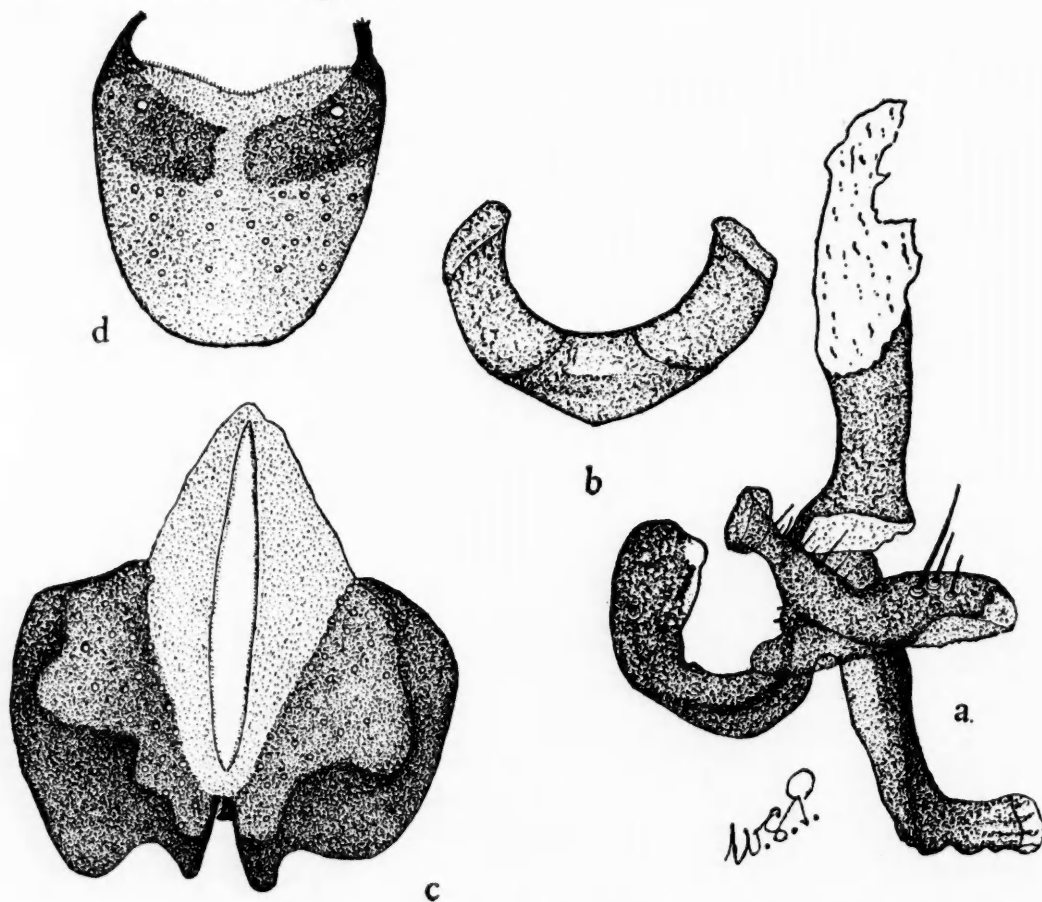


FIG. 24. *a.*—Phallosome and paramere of *M. craggi*—note the rather short chitinous part of body, the well-developed paramere, especially the posterior part, and the long, widely expanded, deeply Y-forked end of posterior process; *b.*—posterior process showing the expansion and marked Y-forking; *c.*—anal cerci—note the well-developed nipples; *d.*—fifth sternum with long lateral process.

CONCLUDING REMARKS

I have now brought forward sufficient evidence to show that the grouping of the species of the genus *Musca* on the following characters has proved to be, not only meaningless, but thoroughly misleading, for by their use species which are not related have been

grouped together, and others which are closely related have been isolated into distinct genera. Further, the grouping as exemplified in the segregates of Malloch fails to explain the true status of the apparently anomalous species, and the blood-suckers, as well as those with a larviparous habit, their relationships to each other and to the rest of the species; these characters are:—

(1) The presence or absence of hairs and bristles on different parts of the body and appendages, and especially on the so-called declivous suprasquamal ridge, the squama and the sternopleuron.

(2) Minor venational characters, such as the closure of cell R_5 .

(3) The presence or absence of minute bristles on the wing veins, especially on the ventral surface of R_{4+5} .

(4) The amount of separation of the eyes of the male.

(5) The glabrous or hairy eyes.

(6) Structure of proboscis.

The comparative study of the male terminalia, on the other hand, has brought out very clearly the following:—

(1) The basic structural uniformity of the parts throughout the species showing how closely they are related.

(2) That there is, therefore, no necessity to split the genus into smaller genera, but only to arrange them in groups according to their relationships, and to indicate the general plan of evolution from the simplest to the highest forms, which will link them up with the related genera in the sub-family Muscinae.

(3) The true relationships of the blood-sucking and larviparous species.

If, then, in this genus of the Muscinae, the characters given above have proved to be not only unreliable but valueless in interpreting the relationships of the species, and the use of which has resulted in an unnatural arrangement of the species and an unnecessary splitting of the genus, the question at once arises, and it is a most disturbing one, as to whether these and similar characters are equally unreliable in the other genera of the Muscinae, and in those of the Anthomyiinae, Calliphorinae, etc. For some years now, innumerable genera have been erected on these characters, and the time is not far distant when we shall have an elaborate classification of the higher Diptera consisting literally of hundreds of genera based on characters such as I have noted above. The higher Diptera

will then be very much in the same state as were the mosquitoes a few years back, when the late Mr. Theobald had completed his elaborate classification of these Diptera on the characters of the scales clothing them. And it must be remembered that scales are only modified hairs, and bristles only elaborate hairs. It will be remembered that the species of one tribe, the Anophelini, were grouped into something like thirty genera on these characters, and there was much confusion regarding the true status of many species, and even genera, of the tribe Culicini, as we understand it now. It was not until Felt, Dyar, Edwards and Christophers studied the characters of the male terminalia from the comparative standpoint, that this unnatural classification was swept away and many genera relegated to the scrap heap of synonymy. And as a result the study of mosquitoes has been much simplified. I fear the same awaits such a classification of any of the larger groups of the higher Diptera, now that we really know the true significance of these terminalic characters in these flies. The studies that are urgently needed to-day are, not so much revision of the species, but revision of genera, for until we really understand the status of many of the genera it is difficult to revise the species satisfactorily. Bristles, hairs and scales, although unreliable guides to the grouping of species, have their undoubted use in their identification, and their use should be limited to this.

As already noted, I have now studied from the comparative standpoint the male terminalia of many typical examples of almost all the well-established genera of the sub-family Muscinae, and hope, as soon as all the illustrations have been executed, to publish a paper in which I shall examine critically the status of these genera from this standpoint. Many interesting and far-reaching results have already emerged from this study. I have for a long time had in preparation a revision of the species of the *Stomoxys* group, which is at present in a most unsatisfactory state, but it was felt that, before publishing this, it was imperative first to examine critically the true status of the many genera erected in this group. I have now examined the male terminalia of many species of seven of the genera, and have come to the conclusion that there is only one genus here, and that that must be the oldest, *Stomoxys*. The male terminalia of these genera are so strikingly alike, the phallosome in particular

being structurally similar in the species, as is the case in *Musca*, and the differences so minute, that I cannot, therefore, see any reason for retaining further any of these genera as distinct except *Stomoxys*, and simply arranging the species into natural groups on the characters of the male terminalia.

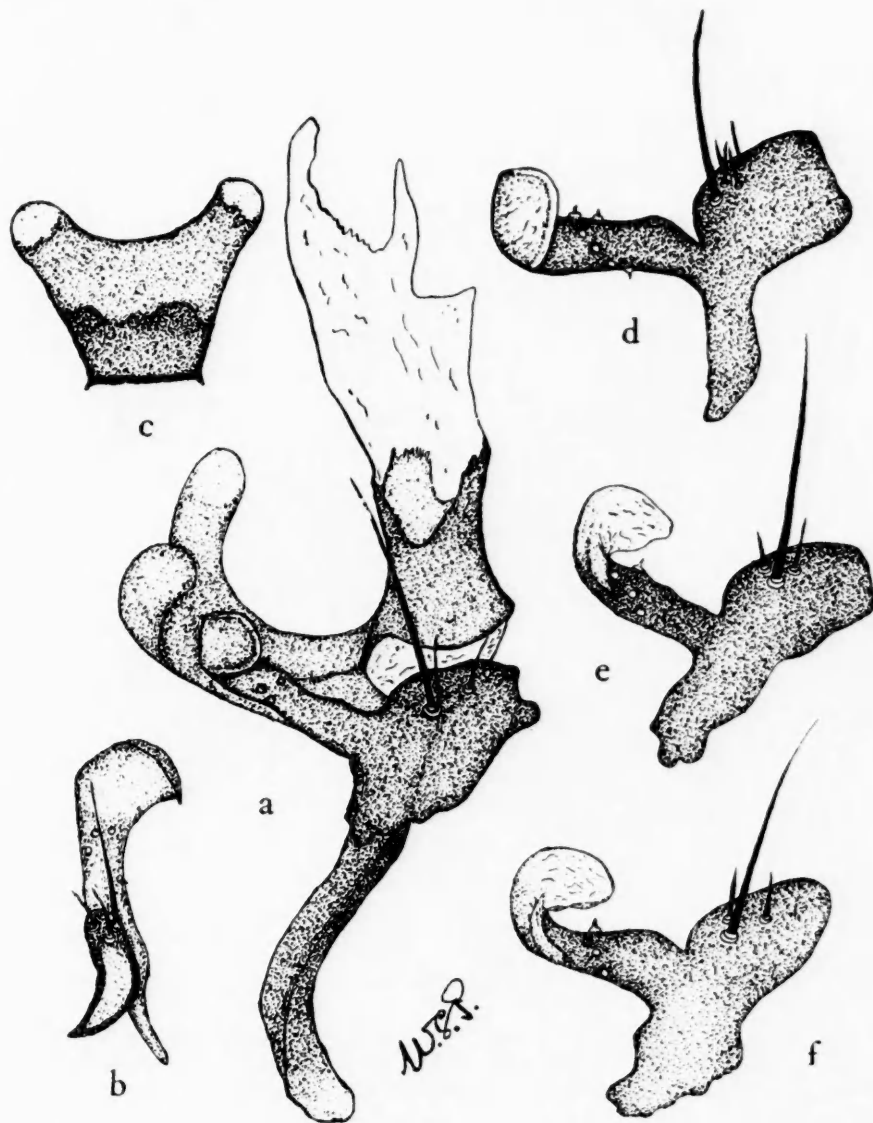


FIG. 25. *a*.—Phallosome and paramere of *M. autumnalis* (*prashadi*)—note the short chitinous part of body, the large anterior end of paramere with three bristles, the posterior part with characteristically lightly chitinised expanded and markedly bent end, and the widely expanded, Y-forked posterior process; *b*.—front view of paramere; *c*.—posterior process showing expanded and Y-forked end; *d*.—paramere of specimen from Southern Europe; *e*.—paramere of specimen from England; *f*.—paramere of *M. prashadi* from Kashmere.

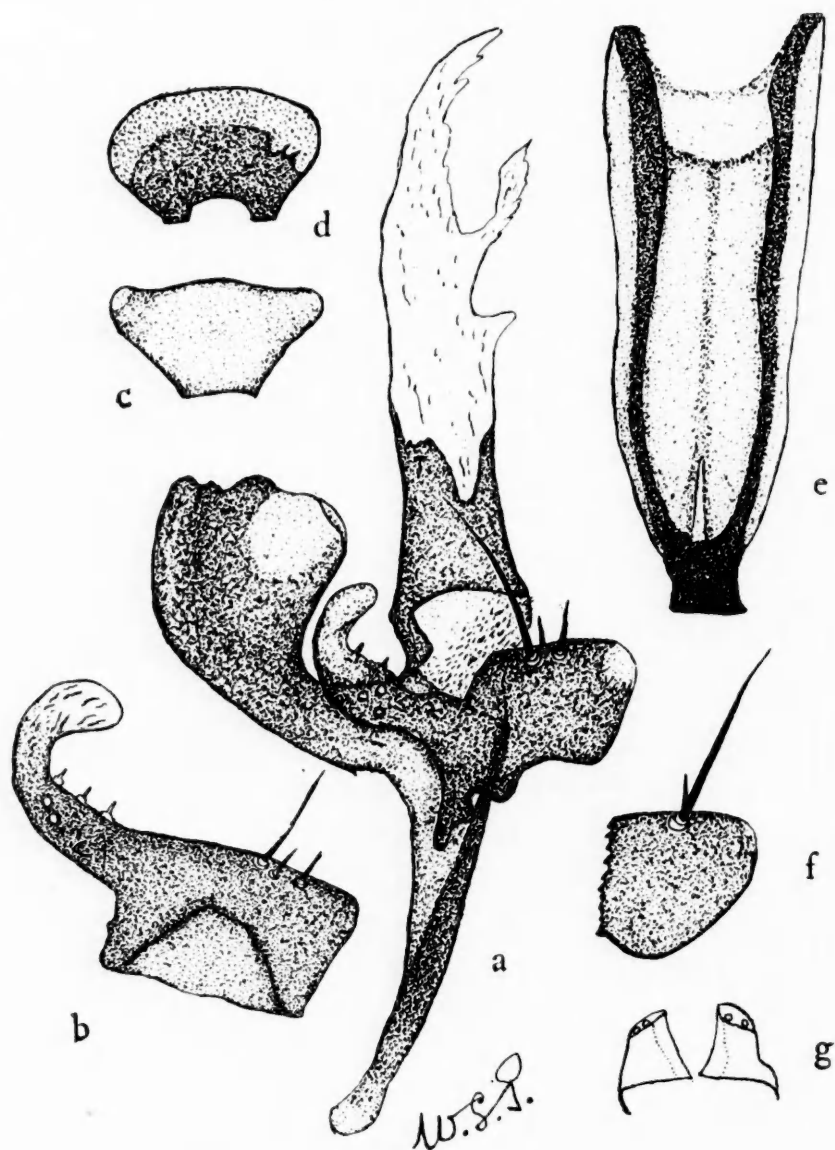


FIG. 26. *a*.—Phallosome and paramere of *M. gabonensis* (*scatophaga*)—note the short chitinous part of body of phallosome, the three bristles on the anterior part of paramere, the short spines on the posterior part, the broadly expanded posterior process, not Y-forked; *b*.—a paramere dissected off to show its structure; *c*.—posterior process seen from back—note its width; *d*.—same from front—note the wide end; *e*.—apodeme of phallosome seen from front—note its characteristic shape; *f*.—anterior part of paramere of another specimen showing only two bristles; *g*.—anterior part of parameres from front.

In order to complete my studies on the genus *Musca* and the *Stomoxys* group, some more material, especially males, of certain species are required. I shall be most grateful if anyone who happens to see this request can help me obtain the species noted. More specimens of the following are wanted for further study :—

GENUS *MUSCA*

Species	Locality where found
<i>Musca lucens</i> Vill., a small species with two thoracic stripes, common about and on animals.	Trincomali, Haragam, Kandy, Ceylon.
<i>Musca albomaculata</i> Macq., a small doubtfully distinct species found about and on animals.	Mauritius.
<i>Musca alpesa</i> Walker, a large haematophagous species with two broad thoracic stripes with a large greyish blue stripe between.	Sierra Leone.
<i>Musca gymnosomea</i> Rond., a small doubtfully distinct species with two thoracic stripes, found about animals.	Malta.
<i>Musca fasciata</i> Stein, a small species with two broad black thoracic stripes.	Seychelles.
<i>Musca dasyops</i> Stein, a dark, almost black, species with two broad black stripes and very hairy eyes. About dung and on foliage.	East Africa; Kilimanjaro; Mount Kenia; Aberdares; Canton, South China.
<i>Musca villeneuvei</i> Patton, a small species with two broad black thoracic stripes, found about elephant dung.	Nilambur, South India.
<i>Musca craggi</i> Patton, a small species with four thoracic stripes, the inner pair narrower than the outer; found about animals and cow dung.	Widely distributed in Oriental region, common in Ceylon and South India and the Philippine Islands.
<i>Musca pulla</i> Bezzi, a small species with four thoracic stripes, the only known specimen is a male.	Pretoria.
<i>Musca albina</i> Wied., the male small and somewhat metallic, the female silvery; a semi-desert species.	Widely distributed in Africa, India and Ceylon.
<i>Musca illingworthi</i> Patton, a medium-sized species.	Java.
<i>Musca fletcheri</i> P & S-W., a large species from cattle.	Samalkota, Eastern India; Shencottah, Western Ghats.

More specimens of *Musca* are required from Sumatra and the Malay States; also from Formosa and Canton, South China.

GENUS *STOMOXYS*

AFRICAN SPECIES.

Species	Locality where found
<i>Stomoxys boueti</i> Roubaud, a remarkably small species.	Agouagon, Dahomey ; Liberia.
<i>Stomoxys bouvieri</i> Roubaud	French Congo ; Dahomey ; Ivory Coast ; Belgian Congo.
<i>Stomoxys inornata</i> Grünberg	A West African species from Liberia ; French Congo ; Gold Coast (Ashanti) ; and Cameroon.
<i>Stomoxys ochrosoma</i> Speiser, a strikingly yellow species.	Kibonoto, Kilimanjaro, Tanganyika Territory ; Mai Iwvi, North of Ruchuru on buffalo ; hills of Luvungi ; all Belgian Congo.
<i>Stomoxys rhodainica</i> Roubaud	Kibati, North of Lake Kivu, Belgian Congo.
<i>Stomoxys sexvittata</i> Roubaud	Banks of Niger between Rarimama and Niamey, French Sudan.
<i>Stomoxys sitiens</i> Rondani	Keren, Abyssinia.
<i>Stomoxys sanguinaria</i> Austen, a little known species, probably crepuscular, biting at dusk.	Katanga District, Belgian Congo ; Monkey Bay, Lake Nyasa.
<i>Stomoxys woosnami</i> Austen, also a very little known species, probably crepuscular in habit. Only ♂ known.	Plateau above Naivasha, Kenya.
<i>Stomoxys praedatrix</i> Enderlein	Bomana, Bibundi, Cameroon.
<i>Stomoxys lutosa (squalida)</i> Grünberg, a brick-coloured species with four narrow reddish stripes, long yellow proboscis, light lemon yellow palps, legs yellow.	Bulia, Tanganyika Territory ; Kenya Colony.
<i>Stomoxys hirudo</i> Enderlein	Kuti, Ntem and Banje Riben, Cameroon.
<i>Stomoxys potans</i> Bezzi, a relatively large species	Eritrea, Italian Somaliland.
<i>Stomoxys shillingsi</i> Grünberg, a small greyish species with yellow legs, yellowish palps as long as proboscis ; abdomen greyish. Common on white rhinoceros.	Donje Erok, Keitloa, Tanganyika Territory ; N.E. Uele District, Belgian Congo.

ORIENTAL SPECIES.

Species	Locality where found
<i>Stomoxys pusilla</i> Austen, a doubtful species. It will be necessary to compare the male terminalia with that of <i>indica</i> . I have not seen specimens from India which I have been able to identify as this species. It will be necessary to have more specimens of <i>Stomoxys</i> from the type locality.	Allahabad, India, in October.

ORIENTAL SPECIES—*continued*

Species	Locality where found
<i>Stomoxys pulla</i> Austen, a very distinct species, only known from the type male.	Mussoorie, N.W.P., India, in October.
<i>Stomoxys triangularis</i> Brunetti... ..	Maddathorai, Pallode, Travancore, South India, in November.
<i>Stomoxys sanguisugens</i> Austen, very close to the European <i>stimulans</i> , and will only be finally distinguished on a comparative study of the male terminalia.	Kasauli, Punjab, India; also at other Himalayan hill stations.
<i>Stomoxys rufipes</i> Brunetti, also very closely related to <i>stimulans</i> .	Darjiling and Noalpur, Nepal.
<i>Stomoxys flavobirta</i> Brunetti, doubtfully distinct from <i>exigua</i> .	Amherst District, Tennasserim, Lower Burma.

In my next paper in this series I shall describe the Palaearctic species of the genus *Musca*. Following this, the Ethiopian and Oriental, and lastly the Australasian species. In each case keys will be given to the identification of both sexes; the diagnostic characters of the larvae where known will be described and illustrated, and there will be many illustrations of characters of the adults of use in identification.

CONCLUSIONS

1. The comparative study of the male terminalia of forty-one species of the genus *Musca* proves these parts to be of a remarkably uniform structure in all, especially the phallosome (penis), thus showing the very close affinities of the species. There is, therefore, no valid reason for splitting the genus into smaller genera.

2. The species fall into three natural groups on the characters of the male terminalia, the posterior part of the paramere exhibiting a remarkable development from a simple plate to a large, upright process (fig. 9). The *domestica* group contains the simplest forms, the *lusoria* the most specialised, and the species of the *sorbens* group occupy an intermediate position.

3. The species with a blood-drawing proboscis fall into the three groups, showing that the blood-sucking habit has been evolved independently in each group along the same lines, and more than once, proof of the real significance of these terminalic characters as a sure guide to the true status of these puzzling forms.

4. The larviparous species fall into two of the groups, the first and the third, and the species (*planiceps*) with the most advanced viviparous habit belongs to the first, showing that simple terminalic characters are quite consistent with a highly specialised larviparous habit (cf. *Glossina*), again proving the great value of the terminalia as a guide to the true affinities of the species.

5. Grouping of the species into smaller genera, and isolating single species into distinct genera (*albina*, *inferior*, *crassirostris*), on such characters as the presence or absence of hairs, bristles, thickness of proboscis, etc., has led to a complete misunderstanding of the true nature of these seemingly anomalous or atypical species.

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THE TRANSMISSION OF *WUCHERERIA BANCROFTI* IN SIERRA LEONE

BY

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That infection with *Wuchereria bancrofti* is common in Freetown is shown in Table I, which is drawn up from the results of different observers published in the annual reports of the Medical and Sanitary Department.

TABLE I

Showing the results of blood examinations for microfilariae in Freetown (compiled from the annual reports of the Medical and Sanitary Department).

Date	Place	No. examined	<i>W. bancrofti</i> infections		<i>D. perstans</i> infections		Page of Annual Report
			No.	%	No.	%	
1913	Hospital*	858	78	9.1	1	0.1	16
1914	"	964	94†	9.7†	—	—	16
1914	Prison	118	14†	11.8†	—	—	16
1923	"	203	29	14.3	7	3.4	64
1930	"	134	26	19.0	3	2.2	20
1930	Hospital*	80	7	8.8	1	1.3	20
1931	Prison	97	20	20.6	1	1.0	Not published
	TOTAL	2,454	268	10.9	13	0.5	

* The Colonial Hospital, or its successor, the Connaught Hospital.

† Probably *W. bancrofti*; see text.

The species of microfilaria found in 1914 was not diagnosed with certainty. The report says: 'There is not sufficient time, owing to the routine work to be done, to work out accurately the type of *Microfilaria* found, but my experience is that the embryos are invariably sheathed and appear to conform to the *Microfilaria* type. It is not common to find *Microfilaria* in the day blood, and since 1911 it has not been my experience to come across or even hear of any of the conditions associated with loa infections.' They have been entered as *Mf. bancrofti*; but possibly in this and other years an occasional *Mf. loa* has been wrongly diagnosed as *Mf. bancrofti*. Such a mistake is very easily made where the two species resemble each other so closely, and one is greatly predominant.

The responsibility for the transmission of this parasite in Sierra Leone has never been fully investigated. Several workers have found nematode larvae when dissecting captured mosquitoes, and have thought that they were dealing with the larvae of *W. bancrofti*, but the truth of this can only be proved by the complementary experiment of feeding mosquitoes bred in captivity on human beings known to be infected with *W. bancrofti* only. In view of the uncertainty, it was decided to investigate the question afresh by breeding mosquitoes from the egg or larva, allowing them to feed on a patient infected with *W. bancrofti*, and dissecting them at suitable intervals after the feed.

In a mosquito survey of Freetown and Kissy (Gordon *et al.*, 1932), *A. costalis*, *A. funestus*, *A. nili* and *A. rhodesiensis* were taken in houses. These were dissected and searched for nematodes. The results of these dissections of wild mosquitoes are recorded in their paper, the present paper being concerned with the development of microfilariae in laboratory-bred mosquitoes. Of the small number of culicines which they captured, *Culex* (*Culiciomyia*) *cinereus* and *nebulosus* were the most common. A few *Stegomyia fasciata* and one female *Taeniorhynchus* (*Mansonioides*) *africanus* were captured.

Several of the local species are mentioned in Edwards' (1922) list of the vectors of *W. bancrofti*. He included *A. costalis* and *T. africanus* with 'Mosquitoes in which complete development has been observed,' *S. fasciata* with 'Species in which partial development only has been observed,' and *A. funestus* with 'Species examined with negative results.'

In the experiments described in this paper the species investigated were :

- A. costalis* Theo.
- A. funestus* Giles.
- A. rhodesiensis* Theo.
- A. squamosus* Theo.
- S. fasciata* Fab.
- C. nebulosus* Theo.

TECHNIQUE

All mosquitoes were bred from larvae collected from their natural breeding places, or from eggs laid in the laboratory by captured females. A strain of *S. fasciata* was maintained for several months. *A. nili* was omitted because no larvae were available at the time of the experiments, while *A. squamosus* was included, as a native collector brought in a batch of its larvae while searching for those of *A. costalis*.

When a suitable number of adults was available, they were enclosed in a small feeding cage and applied to the skin of a patient whose blood was known to contain *Mf. bancrofti* and in whom examination of the thick films had shown no other microfilaria. The application was made at night between nine and ten o'clock and lasted forty minutes. At the same time 20 c.mm. of the patient's blood was spread on a slide, and stained so that the microfilariae might be counted. Next morning the mosquitoes were examined with a hand lens to eliminate those which had failed to feed, and to estimate the size of the meal in the others. Those which had fed were placed in glass cylinders covered with damp cotton wool, and allowed to feed on raisins. The raisins were removed one week after the blood feed, as it was thought that larvae might emerge from the proboscis while the mosquito was feeding on the raisins, and so might evade discovery. In the first three experiments, however, the raisins were offered to the mosquitoes until the latter were killed for dissection.

Mosquitoes were dissected as they died, or were killed and dissected about the fourteenth day after the meal. The head, thorax and abdomen were separated and teased up on a slide, each in a small drop of boiled saline; a cover-slip was placed over each drop and the preparation was examined under the microscope. In the thorax and

abdomen, both mature and immature larvae were seen, according to the interval after the infecting feed, but all larvae in the proboscis were apparently mature, and their presence there has been taken as evidence that the mosquito was capable of transmitting the parasite.

Certain larvae both from wild and bred mosquitoes were fixed and stained for comparison, as described below.

The number of microfilariae in the patient's blood was an important factor in procuring infection. When it was less than 1 per c.mm. of blood, in no case was the mosquito infected; but when it was 10 to 15 per c.mm., a considerable number of mosquitoes became infected without increase in the mortality. This is in contrast to the experience of Bahr (1912), who found that about three microfilariae per c.mm. was the 'most favourable both for the development of the filaria and for the longevity of the mosquito,' while on one occasion, when the number was 13.75 per c.mm., nearly all his infected mosquitoes died. Bahr, however, was working with culicines, whereas most of our work was done with anophelines.

***ANOPHELES COSTALIS* Theo. (*A. GAMBIAE* Giles)**

EXPERIMENTAL INFECTIONS. Annett, Dutton and Elliott (1901), in Nigeria, fed *A. costalis* on blood containing the embryos of *F. nocturna*. On the seventh day, only five mosquitoes remained, but two of these contained in the proboscis mature larvae, measuring about 1.006×0.025 mm. The authors do not say whether the mosquitoes were captured as adults or bred from larvae in the laboratory. In the first case there is the possibility, unlikely though it is, that the larvae were those of some other nematode, perhaps derived from an animal source, before the mosquitoes were captured.

In our experiments we fed 125 *A. costalis*, of which ninety-two survived to the fourteenth day. A number of these showed larvae in the proboscis and elsewhere (see Table II). These have not been expressed as percentages of the ninety-two survivors, as the numbers of larvae found depends in part on the number of microfilariae in the infecting blood, which varied in each experiment. Therefore each experiment must be taken by itself, and in it the number of infections has been expressed as a percentage of the number of mosquitoes surviving to the fourteenth day. It might be said that the infections

should be given as percentages of the total number which took blood, or even of all who were offered the meal, on the ground that longevity and desire for human blood are important factors affecting the powers of transmission of any species. But both longevity and appetite are, in different species, so differently affected by captivity that we do not think they can be estimated, at any rate in the present experiments.

Table II gives the results of these experiments and shows that under laboratory conditions *A. costalis* is an efficient vector. In Experiment 5, mature larvae were found in the proboscis of seven out of thirteen mosquitoes, which were fed on a heavily infected patient and which survived to the fourteenth day. In Experiment 15, however, there is a striking failure, which we cannot explain, of blood rich in microfilariae to infect.

NATURAL INFECTIONS. Larvae thought to be those of *W. bancrofti* have been found in dissections of *A. costalis* by many workers. Ross, Annett and Austen (1900) found all stages of the larva in many of the *A. costalis* caught at Wilberforce Barracks, near Freetown. Annett, Dutton and Elliott (1901), in Nigeria, found them in 5.7 per cent. of 281 *Anopheles* (apparently *A. costalis*). Taylor (1930), in Northern Nigeria, found in 1,936 *A. costalis* that 1.65 per cent. had mature larvae and 6.97 per cent. had immature larvae.

THE CONCLUSION, then, is that *A. costalis* is an efficient and important transmitter of *W. bancrofti*.

ANOPHELES FUNESTUS Giles

EXPERIMENTAL INFECTIONS. *A. funestus* is an unwilling feeder in captivity. Many were bred from larvae, or, when larvae could not be found, from eggs laid by captured females, but few of them could be induced to feed. Among eight mosquitoes fed on heavily infected blood, and surviving to the fourteenth day, only one showed a mature larva in the proboscis, though two other mosquitoes contained larvae in the abdomen and thorax, which were apparently mature. One other case of a proboscis infection occurred in Experiment 4, which is not included in Table II, because it was found that the feeding cage had for some time been in contact with the skin of the operator, a European free from microfilariae, and that some of the

TABLE II

Results of feeding laboratory-bred mosquitoes on blood containing *Mf. bancrofti*.

Species	Experiment No.	No. taking blood	No. surviving to 14th day	Survivors to 14th day				No. of <i>Mf.</i> per c.mm of blood
				Infections all parts		Proboscis infections		
				No.	%	No.	%	
<i>A. costalis</i> ...	1	40	28	5	17.9	1	3.6	1.45
	2	19	10	0	0	0	0	0.8
	3	14	14	4	28.6	0	0	3.1
	5	17	13	9	69.2	7	53.8	13.25
	6	13	11	8	72.7	3	27.3	8.75
	7	2	2	2	100	1	50	10.75
	8	5	5	3	60	2	40	13
	9	1	0	—	—	—	—	0.9
	10	1	1	0	0	0	0	0.35
	12	1	0	—	—	—	—	15
	15	9	7	0	0	0	0	14.75
	17	3	1	0	0	0	0	?
Total ...	125	92						
<i>A. funestus</i> ...	5	1	0	—	—	—	—	13.25
	6	2	0	—	—	—	—	8.75
	8	3	1	0	0	0	0	13
	12	10	7	3	42.9	1	14.3	15
	Total ...	16	8					
<i>A. rhodesiensis</i> ...	7	2	0	—	—	—	—	10.75
	14	1	1	0	0	0	0	15.5
	17	13	6	3	50	1	16.7	?
	Total ...	16	7					
<i>A. squamosus</i> ...	17	7	7	3	22.9	2	28.6	?
<i>S. fasciata</i> ...	7	1	1	0	0	0	0	10.75
	8	2	2	0	0	0	0	13
	9	1	1	0	0	0	0	0.9
	10	13	11	0	0	0	0	0.35
	11	21	17	0	0	0	0	8.2
	12	2	0	—	—	—	—	15
	15	1	0	—	—	—	—	14.75
	19	20	7	1	14.3	1	14.3	?
	Total ...	61	39					
GRAND TOTAL	...	225	153					

* In these experiments, the patient refused to allow examination of his blood.

mosquitoes had fed on his skin before they were applied to the patient harbouring microfilariae.

NATURAL INFECTIONS. Taylor (1930) found that, of 1,260 *A. funestus*, there were mature larvae in 0.87 per cent. and immature in 3.17 per cent. His dissections did not apparently include examination of the proboscis. According to Evans (1927), *A. funestus* has been found infected by Mansfield-Aders in the Zanzibar Protectorate and by Laveran.

CONCLUSION. Though Edwards (1922) includes *A. funestus* in the list of 'Species examined with negative results,' there can be little doubt that it is an efficient vector. That its infection rate is lower than that of *A. costalis* may be due to the fact that it is a smaller mosquito and so takes a smaller meal of blood.

***ANOPHELES RHODESIENSIS* Theobald**

EXPERIMENTAL INFECTION. This species, like *A. funestus*, did not feed readily, and did not survive well in captivity. In Experiment 17, thirty-one *A. rhodesiensis* were offered a blood meal. Four took a full meal, one a half meal, and eight a quarter meal, while eighteen did not feed. Six survived to the fourteenth day, of which one showed a proboscis infection with two mature larvae in the abdomen, while two others showed in each case one mature larva in the head, but not in the proboscis.

NATURAL INFECTIONS. I have not been able to find any record of natural infections.

DISCUSSION. The experimental evidence shows that *A. rhodesiensis* is a potential carrier of *W. bancrofti*. Its efficiency in nature depends on the extent to which it habitually feeds on human blood.

Wood (1915), in the Sierra Leone Protectorate, caught in European or native houses 106 male and seventy-five female *A. rhodesiensis*, and in dissecting thirty-seven of the females found one infected with malaria. Fully two-thirds of the females and practically all the males were caught in his own quarters, having apparently bred and sheltered there during his absence on patrol. Gordon and Macdonald (1930) had great difficulty in finding them in houses near Freetown which were close to the breeding places of the mosquito.

If these results are compared with those of the recent mosquito survey (Gordon *et al.*, 1932), it is evident that engorged females of *A. rhodesiensis* are not caught in houses in the same way as are those of *A. costalis* and *A. funestus*, even when their breeding places are quite close. There are three possible explanations of this:— (1) that it does not readily bite man, (2) that it bites man only in the open, and (3) that it enters houses to bite, but leave soon after its meal, only unfed mosquitoes remaining. There is not sufficient evidence to show which explanation is correct. A single infection of malaria, even if of human origin, does not prove that the mosquito habitually bites man. The fact that the proportion of males to females taken indoors by Wood was high, whereas it is usually low in other species known to bite man, suggests that the mosquitoes may have entered for shelter rather than food, or may be compared with the condition found in *Culex nebulosus* and discussed below. It is significant that, according to Covell (1927 and 1931), no dissections of wild *A. rhodesiensis* are recorded, apart from those of Wood which are quoted above.

Reference to the habits of '*A. rhodesiensis*' as described in Egypt, Arabia and India are misleading, since Christophers and Puri (1931) have shown that it is not the same species as *A. rhodesiensis* of Rhodesia and Sierra Leone.

THE CONCLUSION must be that the mosquito is a potential transmitter, as it allows the development of larvae in the laboratory; but, as the extent to which it takes human blood under natural conditions is unknown, the extent to which it actually transmits the parasite is also unknown.

ANOPHELES SQUAMOSUS Theobald

EXPERIMENTAL INFECTION. As a native collector, when searching for the larvae of *A. costalis*, brought in a batch of larvae of *A. squamosus*, the emerging females were included in the feeding experiment. Of fourteen females in the feeding cage, seven took blood, and all survived to the sixteenth day. Mature larvae were seen in three mosquitoes, of which two had each a single mature larva in the proboscis.

NATURAL INFECTION. Taylor (1930), in Nigeria, found 'immature

infection in which only thoracic muscles are involved ' in 1·2 per cent. of *A. squamosus*.

This species appears to be uncommon in houses in Sierra Leone. It is not mentioned in the lists of mosquitoes caught by Wood (1915), Blacklock (1925) or Evans (1925). The adult is recorded by Gordon (1930) from Mabang, and the larva by Blacklock and Evans (1926), who found it in pools on Kissy flats, where the larvae for the present experiments were found.

CONCLUSION. *A. squamosus* can transmit *W. bancrofti* in the laboratory, but it is not an important carrier in Sierra Leone on account of its rarity.

STEGOMYIA FASCIATA Fab.

EXPERIMENTAL INFECTIONS. Fülleborn (1908) fed four *S. fasciata*, which he had bred from the egg, on a patient harbouring *Mf. nocturna*. On section after nine and ten days no larvae were seen. This experiment, however, is not conclusive evidence against *S. fasciata*, as the number of mosquitoes used is so small, and as the blood of the patient, according to Fülleborn, was only lightly infected.

Bahr (1912) fed laboratory-bred *S. fasciata* on patients having *Mf. bancrofti* in the blood. He reported:—

' All the microfilariae evidently entered the thorax, but only in one instance were they seen to develop beyond a very early stage, although they could be recognised at this stage of development in a lifeless state, as long as seventeen days after the infection. In one individual, however, further development was observed. In this instance, the filariae measured 2·2–3 mm. in length, and possessed a definite alimentary canal; the dissection was made on the twelfth day after the infection. Celloidin sections of a number of infected *Stegomyia fasciata* bear out my statement as regards the inefficiency of this species of mosquito. In only one insect out of the number examined could a filaria be found showing the development of the alimentary canal. In this instance also twelve days had elapsed since infection. It is quite possible therefore that very occasionally development *may* proceed in this species; I never found, however, filariae in the proboscis.'

Vevers (1924), in British Guiana, carried out a similar feeding experiment, using mosquitoes which he had bred from the egg. He

found undeveloped larvae lying in the thorax of two only out of forty-four mosquitoes, thus confirming Low's original work in 1901, when he showed that the species is an unsuitable host.

Table II shows that in our Experiments 7 to 12, and 15, forty-one *S. fasciata* took blood, of which thirty-two survived to the fourteenth day. None of them showed larvae. But in two mosquitoes dying on the fifth and eighth days immature larvae were found. In Experiment 19, in order to trace the fate of the ingested microfilariae, mosquitoes were killed at varying intervals after the meal. The results are shown in full in Table III.

These mosquitoes were derived from eggs laid in a cage by a strain of *S. fasciata*, which had for some time been maintained in the laboratory. The larvae were bred in an open dish on the bench, and the pupae, as they appeared, returned to a cage. To confirm the species, each adult female was examined with a hand lens before being placed in the feeding cage, and again after feeding. The number of microfilariae in the patient's blood is not known, as he refused to allow a blood film to be made, but that it was high is shown by the number of larvae found in the mosquitoes dissected soon after the meal.

The fate of the microfilaria taken up by *S. fasciata* is clearly quite different from that which it meets in an efficient vector. In the latter, according to Stephens and Yorke (1923), after twenty-four hours the majority of the microfilariae have migrated from the stomach to the thorax; at the end of two days, the larvae measure 150μ by 10μ , and on the seventh day, about 225μ by 15μ . In our own experiments, larvae have not been seen in the abdomens of other species when dissected more than twenty hours after the infective feed, apart from mature forms which, however, were not found before the twelfth day. But in the case of *S. fasciata*, larvae remained in the abdomen as late as on the eighth day. Again, the size of the larvae is evidence of abnormal development, for that measured on the fourth day is too large, and those on the seventh too small.

No larvae were found in mosquitoes dissected after the ninth day, until the last was examined, in which were fourteen mature larvae, of which one was in the proboscis. In spite of this, the conclusion must be that the experiments indicate that *S. fasciata* is not a transmitter of the parasite.

NATURAL INFECTIONS. Aders (1917), in Zanzibar, where filariasis is common, says '*Stegomyia fasciata* is ubiquitous and abundant, and this species has shown thoracic but not proboscis infection with *M. bancrofti*.'

Francis (1919), in Charlestown, found that none of the *S. fasciata* which were dissected carried the infection.

TABLE III

S. fasciata fed on blood containing microfilariae and dissected at various intervals after the meal (February 4th, 1932).

No.	Interval after meal	No. of larvae				State of mosquito	Condition of larvae
		Abdomen	Thorax	Head	Proboscis		
1	14 hours	60	21	0	0	Dead	Nearly all alive.
2	"	14	3	0	0	"	All showed lively movements except some larvae in thorax.
3	"	38	7	0	0	"	
4	16 hours	12	3	0	0	"	
5	"	4	3	0	0	Dying	
6	38 hours	0	0	0	0	Alive	
7	"	17	0	0	0	"	All dead.
8	3 days ...	2	3	0	0	"	Alive; thoracic forms sluggish.
9	4 " ...	0	1	0	0	"	Alive; length 235 μ .
10	7 " ...	3	11	0	0	"	Alive, sluggish; in thorax 185 \times 20 μ and 155 \times 20 μ . Abdomen, sluggish; thorax, dead.
11	8 " ...	2	1	0	0	"	
12	9 " ...	0	1	0	0	"	Dead.
13	11 " ...	0	0	0	0	"	
14	14 " ...	0	0	0	0	"	
15	14 " ...	0	0	0	0	"	
16	14 " ...	0	0	0	0	"	
17	15 " ...	0	0	0	0	Dead	
18	15 " ...	0	0	0	0	Alive	
19	16 " ...	0	0	0	0	Dead	
20	16 " ...	8	5	3	1	Alive	All alive and mature except in thorax, where one was immature and alive, and two dead and degenerated.

CONCLUSION. Feeding experiments with laboratory-bred mosquitoes and dissections of wild mosquitoes both indicate that *S. fasciata* does not transmit *W. bancrofti*. The solitary instance, shown in Table III, No. 20, in which a larva reached the proboscis, must be regarded as exceptional.

CULEX (CULICIOMYIA) NEBULOSUS Theobald

C. nebulosus is common in Freetown. In my own house they were frequently found in the ground floor lavatory and sometimes in the first floor bathroom, but never in the bedrooms, which were on the first floor. No engorged females were found, and it was noticeable that males and females were about equal in number.

In the experimental work, some laboratory-bred females were offered a feed on a suitable patient, but all refused, though two *A. costalis* and two *A. rhodesiensis* and one *S. fasciata* fed well in the same cage. We therefore carried out three experiments to determine whether the species would take human blood under more favourable conditions. The mosquitoes were bred from eggs laid in the laboratory. The newly hatched males and females were kept together in damp cages during the day; at night they were liberated inside the mosquito net in which I slept, and collected in the morning. A dish was placed inside the net containing a few raisins, banana slices and cotton wool soaked in water. In the experiment shown in Table IV the same individual mosquitoes were used thus on four consecutive nights. During the day they were kept in damp, dark cages, and were offered raisins and banana. In no case did one of this species bite, though many of them fed on the fruit which they were offered, thus showing that they were hungry. *S. fasciata* was introduced as a control, and fed quickly and copiously. A few mosquitoes were not found in the morning. They were probably among those which died and fell between the net and the mattress, where they were difficult to find. The two *S. fasciata* which were lost were probably crushed accidentally after feeding. Two other similar experiments gave the same results.

On December 24th, the survivors were placed in a glass cylinder which was kept constantly damp. Raisins were offered for food. The first female died on February 8th, 1932; on March 8th, two were still alive. No eggs developed.

Table IV shows that under the conditions of the experiment, *C. nebulosus* preferred fruit juice to human blood. The conditions are that the opportunity of feeding was given (1) on four consecutive occasions to the same mosquitoes, (2) under slightly artificial conditions, (3) at night, and (4) in the dry season, December.

Conditions (1) and (2) are not likely to inhibit a desire for food.

They were offered a feed at night, as there is no evidence that they are a day-biting mosquito. In some houses they are commonly seen in the day resting in dark places, but are not observed to bite.

TABLE IV

C. nebulosus offered a human blood meal inside a mosquito net.

Date	Procedure during day	Species. Only females shown	No. of females in mosquito net			Number of females taking food		
			10.30 p.m.	6.0 a.m. dead	not found	alive	Blood	Juice None
Dec. 20th, 1931	5♂♂, 29♀♀ emerged from pupae	<i>C. nebulosus</i> (unfed)	29	0	1	28	0	3 25
		<i>S. fasciata</i> ... (unfed)	4	0	0	4	4	0 0
21st	In damp cage with fruit ...	<i>C. nebulosus</i> (2 fed)	28	2	2	24	0	14 12
		<i>S. fasciata</i> ... (unfed)	4	0	1	3	3	0 0
22nd	In damp cage; fruit offered to unfed; nothing to fed mosquitoes	<i>C. nebulosus</i> (some fed)	18	0	3	15	0	6 9
		<i>S. fasciata</i> ... (unfed)	4	0	1	3	3	0 0
23rd	As on Dec. 22nd ...	<i>C. nebulosus</i> (some fed)	14	0	1	13	0	2 11
		<i>S. fasciata</i> ... (unfed)	3	0	0	3	3	0 0

They do not appear to undergo in November and December any form of suspended activity or dissociation between feeding and egg-laying. Egg-bearing females were caught at this time of year, and the mosquitoes used in this experiment were bred from eggs laid under natural conditions.

The evidence of other writers is conflicting. Aders (1917a), writing of conditions in Zanzibar, says it is 'an extremely common form, and a very troublesome biter.'

Connal and Coghill (1916), in Nigeria, fed this species on monkeys

with difficulty, and the same authors (1916a), on dissecting 112 specimens, found filaria (species unnamed) in one.

Davis and Philip (1931), using a precipitin test, found chicken blood alone in thirty-eight out of fifty-four tests of this mosquito.

CONCLUSION. It appears probable that *C. nebulosus* in West Africa does not feed on human blood, but comes into houses for shelter. This view is perhaps supported by the large proportion of males captured in my house, with which may be compared the similar condition in *A. rhodesiensis* which was described above.

IDENTITY OF THE LARVAE

The mature larva of *W. bancrofti*, as described by various authors, is about 1.5 mm. long, and 0.02 mm. broad. Bahr (1912) gives 0.9 to 1.6 mm. for the length. The body cavity is lined by a single layer of cells, and contains the intestine running from the mouth to the anus, which is a short distance in front of the posterior extremity. Posterior to the anus the body is rather more slender. There are three anal papillae on the extremity. A short distance behind the anterior end is a break in the column of nuclei, the 'nerve ring.' There is no specific structure by which this larva may be distinguished from similar larvae.

Mature larvae were taken from wild and bred specimens of *A. costalis* and *A. funestus*. Some were preserved in Berlese's fluid or lactophenol, others were fixed and stained. Larvae selected for staining were transferred by a fine needle to a drop of saline on a clean coverslip. The saline was absorbed with blotting paper, and the coverslip was cautiously flooded with an alcoholic fixative, generally Bles. The most convenient stain was haemalum, applied hot, and allowed to act for several hours. All these larvae were found to conform to the above description, and no difference was found between larvae from wild and those from bred mosquitoes. The length was 1.0 to 1.7 mm. and the breadth 0.013 to 0.025 mm. In some larvae, however, it was impossible to distinguish more than two anal papillae.

SUMMARY

1. Human infections with *W. bancrofti* are common in Freetown.
2. Larval nematodes, presumed to be *W. bancrofti*, have often been found in dissections of wild *Anopheles* in West Africa.
3. To determine the vectors of *W. bancrofti* in Freetown, laboratory-bred mosquitoes were fed on patients known to harbour *Mf. bancrofti* in the blood, and were dissected after a suitable interval.
4. *A. costalis* and *A. funestus* both allowed complete development of the larvae. As they are also found infected in nature they must be regarded as important carriers.
5. *A. rhodesiensis* allowed complete development. But as there is no record of its infection in nature and its biting habits are uncertain, it is not possible to decide its importance in transmitting filariasis under natural conditions.
6. *A. squamosus* allowed complete development. It has been found infected in nature, but is an uncommon mosquito in Sierra Leone, and therefore must be considered unimportant as a host.
7. *S. fasciata*. Of thirty-nine specimens which fed on blood containing *Mf. bancrofti* and which survived to the fourteenth day after the meal, only one developed a proboscis infection. This must be considered exceptional, as many of this species have previously been fed experimentally without allowing complete development of the larvae, and as dissections of wild mosquitoes have not shown proboscis infections. *S. fasciata* is not a vector of *W. bancrofti*.
8. *C. nebulosus* appears to be unwilling to bite man. It can therefore be disregarded as a vector.
9. Mature larvae dissected from experimental *A. costalis* and *A. funestus*, and those from wild mosquitoes of these species were indistinguishable from each other, and conformed to the usual description of the mature larva of *W. bancrofti*.

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YAWS AND SYPHILIS

BY

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It is a commonplace that framboesiform lesions in syphilis, comparable to the florid eruptions of yaws among children in endemic areas, have hardly ever been encountered among venereal syphilitics in temperate regions. The yaws eruption is often so striking that many observers are prepared to discard the morphological identity of the spirochaete, the confirmation of the Wasserman reaction, the similar response to arsenicals, in fact, almost everything which usually goes to the establishment of disease identity, and to say that the phenomenon of the eruption alone is sufficient to outweigh the evidence of all these scientific tests. Starting from the outstanding character of the eruption, some writers have elaborated quite a long series of differential criteria to which they attach importance; it is true, these criteria change from decade to decade, but as soon as one is discarded another is found to take its place. The object of the present paper is to examine these differential points as they exist at the present time, and to endeavour to ascertain to what extent they are reliable. Remarkable as are the secondary eruption and certain other of the phenomena of yaws, it appears advisable to try to discover whether there are not possible and adequate explanations of them, before we admit that several of our important and, normally, reliable standards of diagnosis have completely broken down.

Medical officers who go to the tropics and see yaws for the first time, may react in one of several ways: they may call it syphilis with a florid framboesiform eruption; or they may say, as Wallbridge and Daniels (1895) did, that the eruption recalls syphilis at first sight, but on more prolonged observation of the disease, that it is not syphilis and, 'while it is certain they are closely analogous, it is equally certain that they are separate and distinct

diseases.' Or they may, especially if they follow the textbook tabulations of positive differential points, at once take up the attitude that yaws is not syphilis. The predominance of the infection in young children, its non-venereal nature, the absence of grave nerve lesions, and the fact that it is not apparently transmitted congenitally, will all tend to confirm their belief in the existence of two distinct diseases. After a few years experience, however, they will have observed that several diseases affect the natives of a tropical country differently from white people: for example, that an adult native and an adult European in the tropics may react quite differently to infection with malignant tertian malaria, that blackwater fever may frequently result in the case of the white man, but seldom in that of the indigenous native. They will have seen that hookworm infection on a moderate scale may produce severe symptoms in the white, while a very heavy infection in the native in the same region may apparently produce no ill effect; many such divergences in the effects of the same infection on whites and natives will be noted. They may, if the opportunity presents itself, find that what they call yaws has a very florid eruption all over the body in the moist hot low-lying coastal regions, but that in the drier colder highlands it may lose its generalized character, and tend to confine its eruption to the moist warm parts of the body. If they encounter a chancre on the penis, which a man has acquired from a woman with yaws, there will be a difficulty about the diagnosis; they can, perhaps, try to get over it and say, with very little conviction, that, as the man has a venereal chancre, the woman must have had syphilis as well as yaws, i.e. she is a case of yaws and syphilis in the same person. If the man later develops a framboesial eruption, he may also be diagnosed as an instance of syphilis and yaws in the same individual. By this time, however, they will undoubtedly have encountered many other cases which were not so easy to classify as they expected. When they now find that syphilis when it occurs in natives of tropical and subtropical countries shows more florid eruption, that it is frequently not preceded by observed venereal chancre, but often by extragenital chancre, that it does not tend to produce severe nervous system lesions, e.g., tabes and general paresis, and this even in districts where it is stated to be causing a great deal of congenital infection, they may at this stage become somewhat sceptical about

the value of the differential criteria by which they are taught to distinguish two separate and distinct diseases.

The discussion as to the identity or otherwise of yaws and syphilis has for long been rather of academic interest ; recent developments in the treatment of yaws have, however, given a fresh and practical impulse to the subject. By recently introduced methods of treatment, it is possible to get rid of the secondary eruption of yaws easily and rapidly ; now, if yaws is in fact a condition which is closely analogous to syphilis, there appear to be two eventualities to consider in regard to its treatment. An inadequate treatment, which caused the disappearance of the skin eruption while not destroying the infection in the body, might result in a neuro-tropic effect, so that the nervous system might become seriously involved. An adequate treatment, on the other hand, by which the spirochaetes throughout the body were entirely destroyed, might lead to a different, but no less important, sequel. For if yaws infection is capable of protecting against venereal syphilis, then by curing the yaws of children prematurely we are presumably removing their immunity against subsequent syphilitic venereal infection. The prevention of yaws infection in childhood or its cure by successful treatment may thus be a serious step. It is not that syphilis in itself, and in its sequelae as it affects the individual, is any worse than yaws, but because of the congenital infection which results when the spirochaetal attack is postponed to the marriageable age. Congenital spirochaetal infection with resulting intra-uterine deaths and premature still-births introduces the possibility of grave racial deterioration.

It is from this aspect that the evidence should be reviewed afresh, because those who hold that yaws is an entirely different disease from syphilis, will see no danger in the elimination of yaws, whether by prevention or by treatment, whereas not only those who think the diseases are the same, but those also who consider that they are closely related, in the sense that yaws infection protects against syphilis, will regard the wholesale treatment of yaws as fraught with racial danger. They will consider the prevention or cure of yaws, as a positive disadvantage to the people, if its place is to be taken by venereally acquired infection, with its disastrous congenital effects. It may be the case that the congenital effects in the venereal disease of the tropics can easily be prevented, as appears to have

been possible in Uganda from the account given by Cook (1931). If this proves to be so, then one of the main objections to the treatment of yaws would disappear, but until this is proved, those who consider yaws to be syphilis, and those who hold that it protects against syphilis, may well look upon the general premature treatment of yaws as a thing not to be undertaken without further investigation of the possible results.

TEXTBOOK DIFFERENTIAL TABLES

In the vast majority of textbooks which deal with the subject of yaws, there is given a tabular series of diagnostic points by which this disease can be differentiated from syphilis. It may assist us in our discussion if we consider such a list, from a well-known textbook on tropical diseases, and examine the items one by one, in order to discover their approximate value as criteria of differentiation.

I. YAWS: NOT CONGENITAL. SYPHILIS: CONGENITAL

It is useful to take this congenital question first, because, of all the differential items, this is undoubtedly the one which writers on yaws most strongly emphasize, and which consequently deserves the greatest attention.

We may accept as admitted, by most observers, that yaws infection is not transmitted to the foetus *in utero*.

Maxwell (1839) discusses its possibility: 'The yaws is considerably modified during pregnancy, and it is never communicated to the foetus *in utero*. It is very common to see young children at the breast with the disease, but the earliest case which I ever witnessed was the following: A young healthy woman, in her second month of pregnancy, had a scurfy eruption of a pretty general description, unattended with constitutional derangement, shortly afterwards followed by yaws in a mild form, which spontaneously healed before her delivery. Her infant boy, when three months old, was seized with stiffness, appeared to be in great pain, and cried almost incessantly. The symptoms were attributed to a fall, when an eruption of benignant yaws suddenly broke out. At the time this happened there was not a case of the disease upon the plantation, and as the mother was apparently clear of it, the relatives conjectured that it must have been contracted *in utero*; but a more rational explanation would be to suppose that the mother had some remnant of yaws

about the labia, and that it had been communicated to her infant through the medium of a scratch *in transitu*.' We may also refer to the opinion of Wallbridge and Daniels (1897), who say that early congenital syphilitic diseases and the later manifestations such as interstitial keratitis or pegged teeth are unknown in Fiji, where yaws was universal among Fijians and syphilis not known to occur.

Hutchinson (1902) mentions, however, a case of Dr. Powell's : the mother had a breast primary sore and developed an eruption of yaws in January, 1895. In April, 1896, she bore a child which was apparently healthy at birth, but developed an eruption of yaws at seven months. At eighteen months it had a depressed bridge to the nose, prominent forehead, and multiple dactylitis. Hutchinson says that it is much more likely that this case was congenital than acquired. He further points out that Finucane and Corney give evidence that among Fijians notched teeth are occasionally seen, and isolated references to the same phenomena are recorded from other yaws endemic areas.

Now let us look at the opposed statement, namely, that syphilis is congenital, and consider in how far this is true. It appears inadvisable to accept this general statement about syphilis as it stands ; the evidence for it requires to be analysed. Syphilis is not the name of a single, simple and uniform, clinical entity ; on the contrary, it presents so many forms, that it is easily divisible, if we so desire, and in practice it is usually divided, into several distinct clinical categories. We may select a main division of syphilis into two groups, namely, (1) syphilis venereally acquired, with or without chancre, and (2) syphilis acquired non-venereally. This second group may be further subdivided into :—

- (a) Adult syphilis, with extragenital primary sore.
- (b) The syphilis of early childhood with extragenital, or more rarely, with genital primary sore.
- (c) Congenital syphilis with no primary sore, either genital or extragenital.

With regard to our first main division, namely, venereally acquired syphilis, the statement that it is congenital may be admissible, but only if we take care to qualify it by a series of reservations, to the effect that transmission to the partner in marriage does not occur inevitably in venereally acquired syphilis, that the infected

mother may not transmit the disease to her child, that the lapse of even a moderate time from the date of infection of the mother may greatly diminish the chances of its being transmitted transplacentally, that it may be transmitted by her quite irregularly, not to one child but to the next, and that it may even be transmitted to one and not to the other of twins. When we endeavour to ascertain in what percentage of venereally acquired syphilis the disease will be passed transplacentally to the foetus, we obtain figures which are sometimes surprisingly low.

A recent figure by Abraham (1932) is 14·1 per cent. of congenital children in a series of births from ninety-two Wasserman-positive women.

Our second main division, namely, syphilis acquired non-venereally, has for its first sub-division that of adult syphilis with extragenital primary sore, and for its second the syphilis of early childhood with extragenital or, more rarely, genital primary sore. For neither of these two groups have I been able to find adequate figures which are of assistance to us in trying to decide whether such forms of syphilis are commonly transmitted to the foetus. Information concerning the latter category, namely, non-venereally acquired syphilis in early childhood, would have been of the greatest possible importance for our present discussion, in regard to this point of subsequent transmission transplacentally to the foetus. Unfortunately, we have no such figures; the ultimate course of this clinical group of syphilis is not generally known in regard to this point, presumably because the disease is treated as soon as discovered. What the course of the acquired syphilis of early childhood may be, is, and must remain, largely problematical and a matter of speculation, as we may gather from the following opinions in regard to it. Hutchinson (1909) says of syphilis acquired in infancy, ' . . . it still remains a question upon which little or no evidence has been recorded, whether infants who contract primary syphilis in very early life display the symptoms peculiar to inheritance.' Findlay (1919) quotes Gaston's opinion: ' . . . syphilis contracted in the first days of life gives rise to similar malformations, dystrophies and arrests of development to those supervening in intrauterine infection.' Stokes (1927), on the other hand, who uses the word 'congenital' in the strict sense of acquired during the birth process, says: ' Congenital

infections are the more difficult to recognize, *lacking* the "stigmata" of the uterine type, and in many instances are not distinguishable from the infections of extragenital origin, but obscure history, contracted during childhood.' He even considers it probable that infection of the foetus via the blood stream, in the last two months of foetal life, may give rise to the early eruptive pictures and the general symptomatic sequence of an acquired syphilis.

As regards our third subdivision, namely, congenital syphilis, the case is different; there are numerous observations, and they, with few exceptions go to show that this form of syphilis can very rarely be transmitted to the offspring. Whereas McCrae (1930) states: 'The disease can be carried through as many generations as are able to produce'; Hutchinson had said, 'As to inheritance in the third generation, I am absolutely incredulous.' Thomson (1925) remarks 'The transmission of syphilis to a third generation must be extremely rare, but we have it on the high authority of F. W. Mott that it does, sometimes, occur.' Stokes states that 'A healthy child of a heredosyphilitic parent is the overwhelming rule.' Findlay goes further and affirms: 'There is no reputed example of transmission even to the third generation. Like other infectious diseases, it is a pure accident of its environment that the child becomes infected *in utero*.

The experience of the majority of observers has been that trans-placental transmission by congenital syphilitics either does not occur, or does so with great infrequency. Here, therefore, in congenital syphilis, and probably also in the syphilis acquired in early childhood, we have a considerable, and, for our present purpose, a very important volume of the disease, to which the application of the general dictum under consideration, namely 'syphilis congenital,' appears incorrect.

Reverting now to the original statement about yaws and syphilis, it seems to be a fallacy to compare the non-venereal disease of childhood, called yaws, with syphilis in general. We ought at least to refrain from comparing it with venereally acquired syphilis; the comparison should be limited to those forms of syphilis which are non-venereal and which occur in children, namely, the acquired syphilis of early childhood and congenital (transplacentally acquired) syphilis. Since we can discover no sufficient figures as regards the

outcome for the acquired form of early childhood, we are driven back to the congenital form as the best one available, with which we can reasonably and properly compare yaws. Let us now endeavour to make this comparison, and let us put the conclusions we have come to, instead of the textbook antithesis with which we began, namely, yaws never congenital, syphilis congenital. The statement which we now have in its place is this:--*In yaws: congenital transmission very rarely recorded. In congenital syphilis (and probably in infantile acquired syphilis): congenital transmission very rarely recorded.* Thus yaws on the one hand, and congenital (and probably infantile acquired) syphilis on the other, appear to act identically in their effects, in so far as rarity of transplacental infection of the child *in utero* is concerned. This 'congenital' character, much emphasized and often reiterated, is thus seen, when put on the more strictly comparative basis we have adopted, to have little differential value.

But, it may be argued, yaws, although most frequently a disease of early childhood, is not so invariably; older children and even adults acquire it, and why should not they, if the disease is really transmissible congenitally, transmit it to their children? Let us consider the case of the older child first, and take it that we are dealing with a group of boys and girls of the age of eight who have yaws. What will be the after history of the males, and what is the chance of the females of this group at the later time of pregnancy, transmitting the disease to the child *in utero*? The case of these older children, if they had syphilis, would stand midway in time between the congenitally acquired and the venereally acquired infection. We have no adequate figures showing the effect of syphilis, acquired at this age and untreated, in causing transplacental infection, and it seems hardly justifiable to assume that such a type of syphilis would be properly comparable with congenital syphilis in the rarity of its transplacental transmission. On the other hand, we have equally no evidence that it would behave in the same way as venereally acquired syphilis.

Let us, however, for the sake of argument, take it at the worst and assume that yaws, acquired at the age of eight, will normally act in the same way as venereal syphilis would act if it had been acquired at that age. What then should we expect to find as regards

its transmission later to the child *in utero*? Should we expect that it ought to be transmitted by the mother at the age of fifteen or sixteen to her child? Even on the too severe analogy of venereally acquired syphilis I believe we should expect such transmission to be rare. Taking first the males of our eight-year old group, when they reach the age of marriage they will not be able to infect the females of this group, because the females will be already immune; neither will they be able to infect non-immune females, because they themselves, the males, will already have usually passed the infective stage, since the lapse of time factor is of paramount importance even in cases where venereally acquired syphilis is concerned. Thus Harrison (1931) says: 'It is generally agreed that the risk of infection by sexual intercourse becomes very considerably reduced by the end of the second year of infection, and is very slight after five years, though exceptional cases of much longer sexual infectivity have been published. The effect of this is that, on the score of contagion, there is practically no bar to marriage by a syphilitic man after five years.'

Stokes says: 'Time diminishes the infectiousness of syphilis. After five years few cases are infectious.' The same author notes that Keyes placed the chance of infection by the *untreated* husband as 12 to 1 the first year, 5 to 2 the second year, 1 to 4 the third year, and practically nil the fourth and fifth years.

There is a mass of evidence of this nature, the general trend of which is to show that after the first years of the infection, the chances of the venereally infected male passing on the disease to the female, decline with great rapidity.

Turning now to the females of our eight-year group, on marriage they could not infect the immune males, and we would expect to find, owing to the lapse of time, a similar falling off of their chances of infecting non-immune males. But it may be argued that such an infected female might still, though not capable of infecting a non-immune male venereally, be capable of passing on the infection transplacentally to her foetus. On this aspect the evidence points rather to the contrary; Harrison states: 'The transmissibility of the disease certainly becomes less with the age of the mother's infection; thus Belding found that women with infections of more than five years' duration seldom give birth to syphilitic infants.'

Lees' (1927) evidence on this point is similar, 'If the parents suffer from untreated syphilis, the virulence of transmission is always most potent during the first year of infection. It lessens subsequent to this, and a living but weak syphilitic child may be carried to term. By the fifth or sixth years the virulence of transmission slowly lessens, and in many such cases syphilis is no longer transmitted.'

Now the assumption we made by comparing the non-venereal infection of our eight-year old group to the case of venereally acquired syphilis, is making the worst of the prospects. It appears quite probable that a girl who acquired syphilis non-venereally at the age of eight would lose her power of transmitting transplacentally to the foetus even earlier than a woman who acquired syphilis on marriage, and whose uterus was infected from the beginning.

Therefore, I would suggest firstly, that the available evidence is altogether against the probability of children who acquired syphilis at this age being able to transmit it commonly to the child *in utero* later, and secondly that, in this respect, yaws is like syphilis.

So far, we have discussed those females who acquire infection with yaws in early childhood, and those who acquire it midway between birth and the marriage age; these categories represent by far the majority of the cases of yaws.

We come now to the infinitely more limited number of women who acquire yaws at adult age. It is evident that a woman who lives to adult life in a yaws-infected community and escapes the disease, is an exception. There is of course the possibility that a woman may have had yaws as a child and become cured and non-immune, and so liable to a second infection. The adult native woman who escapes yaws in her youth appears to be in much the same position as the adult woman in this country who escapes scarlet fever in her youth. In both cases the woman is liable to acquire the disease from her own child, should this become infected by other children.

The native mother who, having escaped infection in her youth, acquires the disease from her child, would nevertheless, at first sight, appear to be now in a good position to transmit the disease at her next pregnancy. If the next pregnancy were in a year's time, it is probable that she would do so, but in fact her next pregnancy, if there is one, may, among many native tribes, not be in the next

year nor yet in the year after that, owing to the tribal customs and regulations in regard to this matter; here again the lapse of time factor in transmission comes into operation, so that her chance of transmitting would, on the analogy of venereal syphilis, be much diminished by the time her next child was *in utero*.

It will be seen therefore, that the search for instances of congenital transmission of yaws is likely to be rewarded by success among a very limited number of exceptional females, if they are reared in yaws endemic areas. The following series of conditions would require to be fulfilled before such transmission could occur, and before we could have knowledge of it:—

- (1) The mother must herself have escaped yaws in her childhood, a rather unusual thing.
- (2) If she had it as a child, then she must have recovered, become no longer immune, and have then acquired a second attack, a still more exceptional thing.
- (3) Having, however, by some means acquired it, she must then have a pregnancy within a certain time, as she will soon begin to lose the capacity to transmit the infection to the next child.
- (4) These preliminary and necessary, but not highly probable, conditions having been fulfilled, this exceptional woman must now come under the observation of a medical man who takes an interest in the subject of yaws, who understands the nature of the problem, who recognizes clearly what congenital stigmata are reliable in syphilis, and who will not only observe but record the case fully. Incidentally, since it is probable that the signs and symptoms in a child who had congenital yaws would resemble closely or be identical with those of congenital syphilis, there is a danger that the observer, if he is a convinced dualist, will diagnose the case as one of congenital syphilis, because he 'knows' that yaws cannot be transmitted congenitally. If he thus diagnoses the congenitally-infected child of a yaws mother as 'congenital syphilis,' he has vitiated a very valuable observation, owing to a preconceived belief.

All this considerable series of coincidences of facts and observa-

tion of them appears necessary for the discovery and recording of cases of transplacental transmission of yaws ; it is thus not difficult to understand that such transmission may be a fact, and that it may be occurring in all of those few cases in which it is actually possible, and yet very rarely be recorded. A failure to encounter frequently a case of transplacental transmission in yaws need therefore hardly surprise us ; we are looking for a condition which a study of the epidemiology of the disease proves to us must be rare. The fact that we have not found it cannot be regarded as justifying us in denying the actual occurrence of transmission of yaws from the mother to the child ; still less does it justify our making the assumption that such congenital transmission is impossible in yaws.

II. YAWS: PRIMARY SORE EXTRAGENITAL. SYPHILIS: PRIMARY SORE USUALLY GENITAL.

Here, again, we meet the fallacy which results from comparing a non-venereal infection with a venereal one. This highly esteemed differential criterion is no more than the statement of an epidemiological observation ; as a means of differentiating yaws from syphilis it has no significance. By the use of this identical formula, it is open to anyone who desires to do so, to prove that acquired syphilis of infancy in England is an entirely different disease from venereal syphilis. Not only so, but that a large proportion of the syphilis of natives of the French African Colonies, called by the French colonial syphilis, is also not syphilis. Lacapère (1932) points out that among natives, syphilitic venereal chancres are rarely seen, and that the great number of the primary lesions are extragenital. It is of interest to note what this writer calls the three great characters of the syphilitic chancre among natives : ' Il est ordinairement méconnu, son siege est souvent *extragenital* et il se rencontre fréquemment chez l'enfant.'

The extragenital site of the primary lesion of yaws has, therefore, no value as a differential point, by which to distinguish yaws from syphilis.

We may note in passing that the great incidence of extragenital chancre among the natives in the tropics does not appear to be due to any inherent quality of the native skin, which predisposes to it.

The negro in civilized conditions is not more liable to extragenital chancre than white people, in fact quite the opposite appears to be the case. For example, Zimmermann (1921), in the Johns Hopkins Hospital, where the patients were 60 per cent. of them coloured and 40 per cent. white, found twenty-seven cases of extragenital chancre; of these twenty-two were in whites, whereas only five were in coloured patients. Hazen (1914), among a large number of syphilitic negroes, found only one extragenital chancre. Thompson (1920) states that extragenital chancres among the negroes are rare. Reasoner (1916), in order to account for this peculiar freedom of civilized negroes from extragenital chancre, even suggested that there is, in the negro as in the rabbit, a defence mechanism against syphilitic infection outside the genitals.

III. YAWS: TYPICAL YAW PATHOGNOMONIC, FURFURACEOUS AND PLANTAR LESIONS CHARACTERISTIC. SYPHILIS: SELDOM IMITATES FRAMBOESIA.

The 'typical yaw' is presumably a kind of yaw which is commonly to be found in such places as Ceylon and Fiji; yet those who have made careful observations on the disease, hardly seem agreed as to its being pathognomonic. Nicholls (1894) in the West Indies, rejected Kynsey's description and figures of parangi in Ceylon as being the same disease as yaws: 'the conclusion to be drawn from comparing the two diseases is that yaws and parangi are distinct from each other.' Kynsey returned the compliment by saying that West Indian yaws is in reality syphilis.

The diversity of the lesions of the primary and secondary stages of yaws have been emphasized from early times. Maxwell wrote: 'it is well known that the whole children on a plantation may be inoculated from a favourable case of yaws, and that the disease will present as many shades of difference as there are peculiarities among the persons affected.' The early experiments of Charlouis (1881) and others bring out the great variations which may occur, and recent workers like Schöbl (1926) have also shown this. Wallbridge and Daniels were perhaps responsible for the common belief that the yaws eruption was monomorphic. 'The chief distinguishing feature,' they say of yaws in comparing it with syphilis, 'is the uniformity in all essentials of the eruption all through the disease.'

Yet Nicholls, whose report they were discussing, actually described in it three forms of secondary eruption, squamae, papules and granulomata, all three of which may be present at the same time. McLeod (1920) says that cutaneous syphilis in one case may be papular, in another scaly, and in a third framboesiform like yaws. Hutchinson figures in his Atlas an Englishman with a profuse framboesiform eruption of secondary syphilis on the face, and gives reference to other similar cases. He mentions two cases of yaws contracted by Europeans in Africa; in the early stage there was an abundant framboesiform eruption, which had for the most part passed away at the time of arrival in England. In this connection it may be said that primary lesions of syphilis may resemble framboesia: MacKenna (1927) has a picture of an extragenital chancre, showing a raised fungating growth on the lip; if this were present in a native in a yaws country it is difficult to see how one could distinguish it from a yaws lesion.

Such comparisons, however, as those between the eruption of yaws and the eruption of syphilis in Europeans, do not appear to be either necessary or crucial; what is required is to discover whether the eruption of syphilis among unclothed children of the tropics, who acquire the disease by extragenital chancre, differs from that of yaws in children. Apropos of this, there are certain observations which show that even in venereal syphilis there are important differences in the secondary eruption as between the white and the coloured patient, even when the latter is civilized and wears clothes. Stokes points out that the annular secondary syphilide, a form of eruption relatively rare in white patients, is very common in the negro. Zimmermann found that macular and maculopapular varieties comprised approximately 70 per cent. of the eruptions in white patients and 35 per cent. in the coloured. The macular is overlooked more often in the negro, because of the difficulty of detection in the coloured skin. Pustular syphilis, including the follicular and the large acuminate pustular varieties, occurred in 24 per cent. coloured males and 14.1 per cent. black females; only one pustular rash was seen among whites. In a number of negroes the eruption and associated fever and malaise aroused the suspicion of variola. Among two hundred and twenty-eight whites with early secondary syphilis, the annular papular syphilid was only

found on two occasions, whereas in two hundred and seventy-nine negroes it occurred in no less than forty cases. Zimmermann points out that his observation confirms Fox, Gilchrist and Hazen in regarding the annular papular syphilis as a most striking peculiarity in negro syphilis. Gourley (1924), in describing venereal diseases among Indian women and speaking of the types of secondary eruption says: 'One, the papulo-pustular type, needs special mention.' It is not infrequently so pustular as to make one stand back and enquire about the possibility of *smallpox*. These and similar observations suggest that we should find still greater differences in nature; it is necessary, as stated, to study the eruption of secondary syphilis in native children with extragenital chancre and living unclothed in rural areas, in order to obtain a just comparison with yaws.

The furfuraceous lesions are probably thus emphasized because they look more conspicuous on the native skin, where the branny scales are seen against a dark background.

The differential point about the lesions of the feet, is one which should be taken in conjunction with a consideration of the effects of pressure and injury, in causing localization of a syphilitic eruption. If the syphilitic were compelled constantly to walk barefoot on rough ground, e.g., in the case of syphilitic native children, it appears highly probable that the lesions of the feet in such a syphilitic would present much the same characteristic appearance as that attributed to the feet lesions of yaws. It is evident that if a syphilitic developed thick tough skin on the soles of the feet from going barefoot, the bilateral affections of the feet in the secondary stage, which are subepidermal papillary outgrowths, would tend to occur on the soles and be both painful and chronic. In syphilis it is well recognized that the secondary and tertiary lesions are prone to be most evident at places in the body which are subjected to pressure and injury, as are the feet in yaws countries. Taylor (1930) notes that in all stages of syphilis there is a tendency for the lesions to appear at sites of injury, e.g., mucous patches and glossitis of smokers, squamous syphilides on palms of manual workers, gumma of frontal bones of Mohammedans—from striking forehead during worship. Lacapère reinvestigated this last point, after observing the frequency of forehead gummas in the French Colonial natives. He examined healthy Mohammedans and found commonly a pigmented patch or

a serous bursa on the forehead ; this form of injury, he concluded, determines the localization of the syphilitic lesion.

The importance of a multiplicity of skin lesions in causing infection to be easily acquired in the first instance, as an explanation of the extent and probably the character of the secondary metastatic eruption, and of the subsequent wide involvement of the skin in the gummatous lesions of the tertiary stage, is a factor which has not received sufficient consideration in yaws.

Before accepting the criteria dealt with under this heading as being of value in distinguishing yaws from syphilis, the comparison will require to be put on a satisfactory basis. For the same reason that the forehead of the syphilitic Mohammedan shows lesions not to be found in the non-Mohammedan, it is probable that the lesions of the bare feet of the syphilitic native child will differ from those of a well-shod European adult.

IV. YAWS: MUCOUS MEMBRANES NOT AFFECTED. SYPHILIS: MUCOUS MEMBRANES AFFECTED.

As regards the yaws side of this statement, Maxwell long ago expressed his disagreement with it in the following remarks on the eruption of yaws :—‘ Mr. Mason, who writes a sensible Essay on yaws, has fallen into error in supposing “ the mucous tissues incapable of producing yaws tubercle.” That an attack on these membranes is an unfrequent occurrence I am ready to admit, but cases have occasionally come under my observation where the mucous tissues, not only of the throat and nostrils, but also of the ears, were affected, and although much inconvenience was experienced from these delicate parts having been the seat of yaws, they always disappeared with the general eruption, and when the constitution was unimpaired no disagreeable consequences followed.’ Charlouis describes confluent tubercles in the nostril, on the septum, ala and floor. We have also the observation of Hutchinson that while he was in Ceylon he found, in cases produced as typical parangi, that there were present symmetrical filmy sores on the tonsils just like those of syphilis. In more recent times we may quote from Castellani (1922), writing of lesions of the mucous membrane: ‘ These are not very common, but small granulomatous nodules may develop at the base of the tongue, as may whitish patches like syphilis leukoplakia.

Small granulomata may form on the nasal mucous membrane, and also on that of the vagina.' Callanan (1925) records secondary lesions on the tongue.

The conclusions which one reaches from a consideration of the evidence about yaws is that in yaws the mucous membranes are sometimes affected during the secondary stage, although not apparently with any great frequency.

Turning now to the other side of the statement, namely, *Syphilis: mucous membranes affected*, Hutchinson points out that one of the common fallacies about syphilis is the belief that sore throats and infection of mucous membranes are almost an invariable state. He remarks that mucous patches, and ulcers of tonsils, are very often superficial and almost painless. They are often present without the patient knowing and pass away very quickly. MacLeod says, 'Erosions of mucous patches do not as a rule give rise to subjective symptoms, unless cracks are present or when irritated by smoking, alcohol, hot food, hot drinks or highly-seasoned dishes. They are more common in men than women as they are to some extent determined by the use of tobacco and alcohol.' Stokes concludes that, 'Tobacco has been the *bête noir* of infectious syphilis for generations. There can be no reasonable doubt that its irritant effects promote the appearances of the infectious lesions in the mouth, and may greatly prolong the period of danger to others. Syphilitic patients, in the first five years of the disease, should give up smoking and chewing.'

Zimmermann, it may be noted, found secondary lesions in the mouth and pharynx to occur in 42.1 per cent. of white patients, as compared with 27.2 of the negro patients in his series of cases. He refers to the mutually contradictory statements of Hazen and Baetz on this subject, and also to the observation of Carter, who did not observe a single mucous patch in two hundred and thirty-one coloured syphilitics, while such lesions occurred in twenty-one of a similar number of white patients. It appears probable that, if there is a smaller percentage among adult negroes than among adult whites, the reason may be sought partly in a relative absence of causes of injury and irritation of the mucous membranes in negro patients.

If we admit that mucous membrane lesions of the mouth are

determined by irritants, we should not expect such lesions to be common in yaws, because these children are not subjected to such irritations of the mouth, as tobacco and alcohol, which are recognised by all syphilologists as especially predisposing to lesions of the mucous membranes of the mouth.

There is one important observation on this problem, which has been emphasised by Hutchinson and confirmed by several subsequent authorities. Speaking of inherited syphilis, he observes: 'The relapses frequently seen in adults who have acquired the disease find no place in infants, nor do we meet with the troublesome affections of the mucous membranes, sore throat, sores on the tongue, etc., which so often happen in them.'

The evidence thus shows that it is not correct to say that in secondary yaws the mucous membranes are not affected, and that it is equally incorrect to suggest that the mucous membranes are always affected even in secondary venereally acquired syphilis. If we are seeking to institute a comparison between yaws and syphilis, we require to know how the mucous membranes are affected in native children of the tropics who acquire syphilis with an extra-genital chancre. Such a group would appear to be the only fair one for comparing syphilis with yaws in children.

V. YAWS: ITCHING COMMON. SYPHILIS: ITCHING RARE.

If we consider the primary lesion in yaws and syphilis, the evidence that this criterion of itching will assist us in our diagnosis is not very convincing. Maxwell talks of the intolerable itching which occurs in some cases in a precursive eruption of minute papules, which is one of the eruptions which he found to precede the primary lesion of yaws; about the primary lesion itself he has little to say of itching. Castellani says there may be some pruritus in the primary lesion. Callanan notes that in the early stages it may be irritable. Of the primary sore of syphilis, Taylor says, the first sign is a small red itching papule. MacLeod records that it may itch slightly at first. There are many other records of this inconclusive nature both for yaws and syphilis.

With regard to the secondary stage of yaws, Castellani says, 'The principal type of eruption in yaws is a papule, which proliferates

into a characteristic framboesiform granulomatous growth ; there is extremely well-marked pruritis.' Callanan notes that some itching of the secondary eruption occurs. Of secondary syphilis, Taylor says that itching is uncommon but that this feature is not constant.

It is of interest to find that Zimmermann states that pruritus is unusual in secondary syphilis in white patients, while it is not infrequent in coloured patients, especially in connection with the follicular syphilids. On this subject Branch (1907) says: 'Absence of itching is admitted to be by no means an absolute rule in syphilis ; nor is itching at all the rule in yaws.'

It will be noted that there is no agreement such as would enable us to say that itching constitutes a real point of differentiation, either as regards the primary lesion or the secondary eruption. In many cases, the skin of yaws-infected persons is infected with scabies ; further, the number of lesions on the naked skin of yaws children, caused by biting-flies, penetration of larvae, cuts and abrasions, is enormously greater than is ever the case in adult Europeans ; so that itching infections of the skin, quite apart from spirochaetal lesions, are very much more common in them. This fact undoubtedly accounts for a considerable amount of itching of the skin in native children whether they are infected with yaws or not. Itching is in reality almost a property of the skin of the native child, and it is difficult to see how it will be possible to decide what part of any itching we find in infected native children can properly be attributed to yaws lesions, without first eliminating the numerous causes of itching from which they suffer quite independently of any yaws infection.

VI. YAWS: ALOPECIA NOT KNOWN. SYPHILIS: ALOPECIA MAY OCCUR.

Callanan, speaking of yaws, notes that the eruption may produce localised alopecia. Hutchinson says that syphilitic alopecia is by no means common—a diffuse thinning on the scalp is not uncommon, but it rarely approaches baldness.

In relation to this question of alopecia, it has to be borne in mind that those who are infected with yaws are natives of tropical countries and mostly children, and that among them, whether

owing to their habits as regards headdress or for other reasons, baldness from any cause is rarely seen. Baldness, whether localised or general, is a not unusual attribute of the head of the white man, but hardly of that of the native child.

VII. YAWS: EYES UNAFFECTED. SYPHILIS: IRITIS COMMON, CHOROIDITIS AND RETINITIS RARE.

The iritis incidence presents anomalies for which the explanation does not appear obvious. As far as yaws is concerned, some cases have been recorded from endemic areas; even among the Fijians, who were not supposed to suffer from syphilis, there is recorded the occurrence of occasional cases. As regards its frequency in syphilis, there are various statements; thus Hutchinson says at one time that iritis is fairly common, but later says that he had not seen a case for several years. He states: 'When the secondary eruption is at its height, or just when it begins to decline, there occurs in exceptional cases an inflammation of the eye; the iritis, when it happens, is usually symmetrical.' McCrae says iritis is common in syphilis three to six months after the chancre. Stokes, however, writes: 'Iritis is usually a late secondary; the incidence in our series did not exceed 3 per cent. in the general run of cases; it seems to be more frequently an accompaniment of severe infection.'

We may now refer to certain anomalous findings as regards the coloured patients in America. Whereas in the matters of mucous membrane lesions, florid eruption, and absence of neurosyphilis, e.g. tabes and paresis, the syphilis of the civilised negro stands in an intermediate position between syphilis in the white and yaws, this does not appear to be so in the matter of iritis; incidentally, it may be noted below that the iritis in whites is not high. Zimmermann found only four cases of iritis among two hundred and twenty-eight cases of white patients with early secondary syphilis, whereas there were thirty-six cases of acute iritis among two hundred and seventy-nine black patients, and he notes that in seventeen of them it was associated with follicular syphilids. He states that Atkinson found eleven cases of iritis among one hundred negroes with primary and secondary syphilis. In the tertiary stage he found the negro more prone to iritis than the white; of forty cases in which this

occurred as an isolated lesion, or in association with other tertiary lesions, twenty-nine were coloured patients.

It is possible that further study of iritis in natives in their own countries may throw light on this question ; the incidence of iritis following extragenital syphilitic infection in native children and that resulting from venereally acquired syphilis could be compared.

VIII. YAWS: VISCERAL LESIONS ABSENT. SYPHILIS: VISCERAL LESIONS OCCUR, i.e., PERICELLULAR CIRRHOSIS, GUMMA OF LIVER, KIDNEYS, etc.

Lacapère, in summing up the differences which distinguish the syphilis of natives of the French Colonies both in the Far East and in Africa from European syphilis, says that one of the characteristics of the former is the great rarity of visceral lesions. Gougerot (1932) discusses the peculiar evolution of colonial native syphilis, and he too emphasises the rarity of visceral lesions in this native syphilis. Glück (1904) studied endemic syphilis in Bosnia and Herzegovina, where there was a great tendency to extragenital infection during infancy and childhood ; he noted as one of the peculiar characters of this type of syphilis that there was comparative infrequency of visceral syphilis.

Now if we permit ourselves to use the absence of visceral lesions in yaws as a fact which entitles us to declare that yaws and syphilis are different diseases, we encounter a difficulty. We cannot argue in this way, without admitting that the endemic syphilis of Bosnia and Herzegovina, where children acquire the disease by extragenital chancre, and also the colonial syphilis of the French, are equally not syphilis. But, as we shall see, all the evidence obtained from the observation of Europeans who have contracted syphilis from natives in the tropics, goes to prove that the colonial syphilis is not to be distinguished in any way in its effects on these Europeans, from syphilis contracted venereally from Europeans.

Yaws, as regards its visceral lesions, ought properly to be compared with syphilis in the tropics occurring among children who have acquired it by extragenital chancre, and the evidence suggests that, when this is done, no difference may be found.

**IX. YAWS: NERVOUS SYSTEM NEVER SERIOUSLY AFFECTED.
SYPHILIS: NERVOUS SYSTEM PRONE TO INFECTION,
TABES, G.P.I.**

We are here not dealing with all involvements of the nervous system, so that paraplegias, hemiplegias and monoplegias, which also occur in yaws areas, are excluded from consideration. The discussion under the head of the nervous system is, by the above formula, restricted to the occurrence of tabes and G.P.I. as evidence of serious nervous involvement.

It is not by any means admitted that such conditions are absent as a result of yaws; Harper (1916), in Fiji, where syphilis among the native Fijians was formerly reputed to be non-existent, describes three cases of tabes, and three cases of G.P.I. in Fijians. From other countries, where yaws is endemic, a few cases have also been ascribed to the effects of this infection. Harper says: 'Probably the case rate following yaws might not greatly differ from the case rate following syphilis.' The evidence of this, however, is lacking, and the majority of observers have failed to find tabes and paresis in yaws areas, in anything like the percentages usually given for syphilis at home.

A few examples of the incidence of paresis and tabes in white people at home may be given, and incidentally it will be noted that various observers give very different figures. Part of the discrepancy is doubtless due to the improved methods of diagnosis, to the effects of the cases having had different amounts and kinds of treatment, and to other factors. McCrae gives an account of Mattauschek and Pilz' results in four thousand one hundred and forty-three cases of syphilis followed for from twenty to thirty years. Paresis supervened in 4.7 per cent., tabes in 2.7 per cent. In an epitome in the *British Medical Journal* of February 2nd, 1929, an account is given of the sequels of untreated syphilis; Dahlström gives figures showing that it is estimated that 4 per cent. of the cases develop paresis. Boeck, in a total of two thousand one hundred and eighty-one cases, gives paresis 0.6 per cent. and tabes 0.27 per cent. Zimmermann found paresis in 4.5 per cent. in his series of whites, and tabes in no less than 22.3 per cent.

There are numerous records which suggest strongly that various

factors appear for some reason to determine the incidence of these two sequelae of syphilis. Among such factors we find three which we may select for our present purpose ; these are occupation, race and the character of the secondary eruption.

Occupation. Osler and McCrae (1928) pointed out that the civilized peoples are those who get paresis, and further, that it affects men much more frequently than women ; they state there is now ample evidence that negroes in civilized countries do not escape, and they give figures for New York, comparing them with Cochin China figures. It may be noted in passing that the peculiarity of the Cochin China figures can hardly be ascribed solely to occupation ; they introduce also the factor of race. Lacapère, in Morocco, considers it probable that it is what he calls 'intellectual trauma' which determines paresis in educated Europeans ; he finds difficulty in accounting for tabes, the native being self-indulgent sexually, and he suggests that another factor comes in here, namely, hypertension in the case of the European, which is absent almost always in the native.

Race. Stokes says that it is generally conceded that uncivilized races show a lesser tendency to develop the degenerative lesions of the nervous system than the civilized white races. In American negro types, the preponderance of aortitis and aneurysm and the excessive rarity of tabes dorsalis and paresis are commonly mentioned. Moore (1921) has observed that early neurosyphilis is about twice as frequent in the white as in the coloured race in America ; of three hundred and seventy-seven white patients examined by spinal fluid tests, 15.9 per cent., whereas of two hundred and sixty-five negroes, 8.3 per cent., showed pathological changes in the spinal fluid. Zimmermann found paresis in only 0.8 per cent. of his coloured patients against the already quoted 4.5 per cent. in whites, and tabes in 3 per cent. of the coloured against the 22.3 in the whites. This observer adds somewhat significantly: 'The negro, in whom the occurrence of tabes was at one time the exception, yearly enters more and more into the competition of modern life, and is losing his freedom from this form of syphilis.' He concludes that, in respect to syphilitic infection, there exist inherited biological differences between white and negro patients: the negro develops intense reactions on the part of cutaneous and osseous structures, and is relatively free from tabes and paresis ; in white patients, syphilis

more frequently runs its course with skin manifestations slight or absent, but there is a greater tendency towards the eventual development of tabes or paresis.

Against the argument that the absence of tabes and paresis is a racial effect due to the coloured skin, we may observe that this can hardly be an effective explanation in the case of the endemic syphilis of Bosnia and Herzegovina described by Glück, which is also characterized by a relative absence of tabes and paresis.

Character of the eruption. It has frequently been observed that in syphilis among civilized whites, there is less tendency for neurosyphilis to develop in those who suffer from florid eruption. Stokes refers to this in the following terms :—‘ The now well-substantiated clinical observation that patients who develop marked cutaneous symptoms are relatively immune from neurosyphilis, and that patients with neurosyphilis can seldom give a history of marked skin manifestations, has been used as evidence that each type of manifestation was produced by a separate strain, though susceptible of other interpretations. Just where the influence of the strain of organism ends, and the susceptibilities of the host begin, it is impossible to define with our present knowledge.’ Harrison makes reference to the same phenomenon in discussing treatment : ‘ The rapid effect on clinical lesions led to the belief, which still prevails in some quarters, that one or two doses of ‘ 606 ’ or ‘ 914 ’ are sufficient to cure any case of syphilis. Such a belief can be positively dangerous when put into practice, since the brunt of the relapse may easily fall on the central nervous system, as will be mentioned later on in dealing with neuro-recurrences.’ This author (1931a) showed that of the army treatment cases who had had more than seven injections of an arsenical compound, very few developed neurosyphilis. His explanation is of interest : ‘ Arsenobenzene compounds stop more completely than do any others the inter-action between the skin and mucous membranes on the one hand, and *Sp. pallida* on the other, and hence the development of protective bodies which, reaching the meninges, would have prevented or at least restrained the activity of the spirochaetes there. On the other hand, arsenobenzene compounds admittedly reach *Sp. pallida* in the meninges less easily than they do in skin and mucous membranes, so that a given dose damages spirochaetes in the

external structures more severely than those in the meninges, and these are the first to revive. If this theory is accepted, it is not difficult to see that comparatively large doses of an arsenobenzene preparation may favour neuro-relapse more than smaller ones because the latter may still permit the process to simmer on in the skin and mucous membranes. The value of the concurrent use of mercury or bismuth is that these, unlike the arsenobenzene, remain in the tissues and so restrain the development of syphilitic processes in the meninges.'

It appears from these remarks and observations that a florid skin eruption, allowed to run on for a considerable time, may have a decided effect in preventing the later occurrence of neurosyphilis. Having seen that the three factors selected, namely, occupation, race and character of eruption, appear to have importance in relation to the development of paresis and tabes, let us turn from syphilis as it occurs among Europeans, Americans and American negroes, and consider it as it occurs among native peoples in their own lands.

The figures as regards paresis and tabes in syphilis in the natives of tropical and subtropical countries are remarkable. Baetz (1914) did not find a case of tabes and only one case of paresis in twenty thousand admissions of negroes in the Canal Zone. Fletcher (1922), who worked in Malay, says that it is the experience of most writers in the tropics, that symptoms of early involvement of the nervous system are uncommon in natives, and that tabes and general paralysis are comparatively rare, at any rate among the native population. Harper Nelson (1931) records that in Lahore, during twelve years in charge of sixty medical beds, he saw only two cases of tabes dorsalis and that one of these had European blood, and only one case of G.P.I., and that an Anglo-Indian. Of particular interest in relation to this question are the Uganda figures, because there, among the Baganda people, syphilis is said to be very rife, and congenital syphilis to constitute a grave problem. Cook (1931) says: 'In Uganda it has been an amazing thing to me to see how few cases there were of locomotor ataxy and dementia paralytica. I have always tried to take a careful interest in nerve syphilis. I must have seen considerably over one hundred thousand cases of syphilis, but I do not remember seeing a single case of locomotor ataxy or G.P.I. in Uganda.' Coia (1931) states that in British Guiana, with one hundred thousand negroes and

one hundred thousand Indian immigrants, there have been in ten years two cases of G.P.I. and one of tabes. Decrop (1932) found only two cases of tabes and two of G.P.I. in five years among eighty-four thousand syphilitics in Tangiers.

There is a large volume of evidence to the same effect, and so certain are observers of their being concerned with an actual phenomenon, that several of them have endeavoured to explain the reasons for it. One of the most usual explanations is that of Levaditi and Marie, mentioned by Fletcher: they consider that the relative scarcity of tabes and paresis in syphilis in tropical countries is due neither to racial immunity nor to climate as such, but to the lack of the neurotropic variety of *Spirochaeta pallida*. This line of argument is tempting, but does not appear to be supported by facts; clinical evidence on the contrary controverts the 'strain' theory. Thus Fletcher concludes the remarks already mentioned, about the rarity of nervous involvement in natives, by the significant addition 'but not in European immigrants.' Cook, while he found no cases of G.P.I. or tabes in about one hundred thousand Uganda syphilitics as already recorded, nevertheless observed these sequelae in quite a number of the far less numerous Europeans there. Harrison points out that Europeans infected by native women develop tabes and G.P.I. just as much as do Europeans infected by European women. Gougerot finds that Europeans who contract syphilis from natives who suffer from 'colonial syphilis,' develop grave nerve lesions such as tabes and G.P.I., just as do those who contract syphilis in France. Lacapère has collected no less than three hundred and forty-three cases of Europeans who contracted syphilis from native women in the French African colonies; in all these the syphilitic infection behaved just as it does in cases contracted in France.

In view of this large amount of evidence from such varied sources, the 'strain' explanation does not appear to be either satisfactory or tenable.

Lees (1931) has put forward an interesting suggestion. Referring to the acknowledged and striking relative absence of tabes and paresis in syphilis in the tropics, he does not think it is a case of self immunity; he argues that as the average age of death in India is forty to forty-five, and as the largest number of cases of paresis and tabes in this country develop these complications at or after this age,

this is a possible explanation of the low incidence of tabes and paresis recorded in syphilis from India and the East.

For the purpose of our present discussion, which is the relationship of yaws to syphilis, it will be recognised that this suggestion of Lees, even should it prove to be correct, is not of material importance. Because, if it is true that natives of tropical countries do not live long enough to develop tabes and paresis as a result of syphilis, exactly the same argument can be used to explain why they fail to develop these complications as a result of yaws. We come back now to the criterion under discussion, by which we are to distinguish yaws from syphilis, namely, the nervous system criterion, the statement being : *yaws: nervous system never seriously involved. Syphilis: nervous system prone to infection, i.e., tabes, G.P.I.*

It is evident from all the varied records given above that in actual fact syphilis, as it manifests itself in many parts of the subtropics and tropics among native races, hardly ever causes tabes and G.P.I., whatever the explanation of this phenomenon may be. It is not practical, therefore, to make use of the almost entire absence of tabes and G.P.I. in yaws as a means of differentiating it from syphilis. Yaws may perhaps be distinguished thus from syphilis as it occurs in white races, but it cannot be distinguished thus at all from syphilis as it occurs in native races in the tropics. This is not to be taken as implying that syphilis in natives is any other than syphilis in Europeans; on the contrary, all the clinical evidence points to their being identical infections due to the same spirochaete. The conclusion therefore must be that it is not possible to distinguish yaws from syphilis by the lack, in yaws, of severe affections of the nervous system, namely, tabes and paresis.

**X. YAWS: NO ENDOTHELIAL PROLIFERATION AS IN SYPHILIS.
SYPHILIS: ENDARTERITIS OBLITERANS OF VISCERA, CEREBRAL
THROMBOSIS.**

In regard to the histopathology of yaws and syphilis, it appears that sweeping conclusions have been arrived at as the result of the examination of a very limited amount of material. Furthermore, there is introduced a great source of fallacy in many of the so-called comparisons of the pathological results of infection. For instance, one finds a comparison made between a syphilitic, venereal, indurated

chancre, and a yaws primary lesion. Now there are many reasons why such a comparison should be regarded as invalid. An indurated venereal chancre in an adult with syphilis does not appear properly comparable with a non-indurated extragenital lesion in a child with yaws. It does not require the use of the microscope and the examination of sections to prove that very great differences exist between these lesions: mere inspection and palpation tell us that. I would suggest that many of the conclusions reached are based on such comparisons of the wrong material. If we wish to compare the extragenital primary lesion of yaws in a child with any lesion in syphilis, it should clearly be with an extragenital primary lesion of equal duration in a child of approximately the same age, of the same race, in the same environment. For an indurated genital chancre in an adult male with syphilis, the comparative object should be, if obtainable, an indurated genital chancre in a male acquired from a yaws infected female.

It has been shown that in syphilis in natives of the tropics, tabes and paresis may be extremely rare; there is, however, a considerable incidence of aneurysm, and para- and hemi- and other plegias. Harrison, in referring to these lesions in syphilitic natives of the tropics, says: 'Should we not at once say that hemiplegia and paraplegia are not really neuro-syphilis; they are vascular syphilis, and should be classed with the cardio-vascular lesions, the difference being that the vascular lesion is in the central nervous system.'

Since in some yaws endemic areas where venereal infection rarely appears to be present, aortitis, aneurysm and various plegias are yet common, we must either conclude that these lesions have a different pathology from those of syphilis, or else admit that in yaws, just as in syphilis, endarteritis and thrombosis occur.

A further point to be considered while dealing with this question of endothelial proliferation is the histopathology of the gumma. Gummatous lesions of the skin and subcutaneous tissues are usually held to be very numerous in yaws endemic areas. The frequency of gumma of the skin in yaws patients appears explicable on the theory of the origin of the syphilitic gumma mentioned by MacLeod. 'It is believed that the gumma occurs on the site of a previous early syphilide which has involuted and left foci of micro-organisms, possibly about the capillaries, which, after remaining in a quiescent

state for years, for some reason or other renew their activities.' The gumma of syphilis is considered by most observers to involve endarteritis as a precursor of necrosis. Mallory (1925) says the term 'gumma' in syphilis is practically restricted to those tertiary lesions in which necrosis has taken place as a result of obliterating endarteritis. Stokes writes of the cutaneous syphilitic gumma: 'In the pathology of these lesions one sees the granuloma of syphilis most typically portrayed—the giant cells, the lymphocytic infiltration, some epithelioid and fibroblastic proliferation, peripheral obliterative endarteritis of the finer capillaries and arterioles, and central softening and necrosis.'

If we accept the general testimony that gumma of the skin and subcutaneous tissues is common in yaws, then we are obliged either to admit that obliterative endarteritis does in fact frequently occur in yaws, or else to postulate that the histopathology of the cutaneous gumma of yaws is entirely different from that of the cutaneous gumma of syphilis.

XI. YAWS: BETTER RESISTED, CONSTITUTIONAL DISTURBANCES SLIGHT. SYPHILIS: ATTACKS CONSTITUTION, AFFECTING VITAL STRUCTURES.

It is difficult to understand the significance of this statement. The primary lesion of syphilis is in itself commonly a much less troublesome lesion than that of yaws. Most observers will consider the secondary stage of yaws, with its precursor malaise, bone pains and general symptoms, to be at least as constitutionally incapacitating as this stage of syphilis. As for the tertiary stage, a great many people will agree with Branch when he says that if syphilis and yaws are different diseases, then yaws is the worse of the two.

If it is the grave effects on the nervous system, e.g., tabes and general paresis, which are meant by the phrase 'affecting vital structures,' the reader may refer to heading IX where these nervous system lesions are discussed, and where it is shown that the rarity of these affections in yaws does not distinguish it from syphilis among natives of the tropics. Again, if it is the effects on the viscera which are meant, the reader may be referred to the argument under heading VIII where it is shown that the lack of visceral lesions in yaws is

paralleled by the lack of visceral lesions in syphilis in natives of the tropics. Finally, if the vital structures are the blood-vascular system, changes in which cause the lesions which result in paraplegia, hemiplegia and other plegias, reference to them will be found under heading X where it is noted that such lesions are common in yaws endemic areas.

XII. YAWS: DOES NOT RESPOND TO MERCURY. SYPHILIS: RESPONDS WELL TO MERCURY.

Maxwell makes the strongest statement I have been able to find on the result of treating yaws by means of mercury. He is comparing sabbens with yaws, and says :—'Sabbens yields readily and is cured by mercury, yaws is invariably rendered worse by that mineral.' His view is not supported by subsequent workers. Thus, Charlouis, who did not believe that syphilis and yaws were identical, nevertheless says the best treatment for yaws is the combined use of mercury and potassium iodide, and that most authors agreed that the disease framboesia yields to mercury. Iodide of potassium alone had no effect on the tubercle, but the bone pains and constitutional symptoms were relieved by it. Mercurial ointment was applied to the tubercles and such treatment cured the disease within one or two months (seldom longer). 'Even Sauvages (who denies the syphilitic nature of framboesia) and Plenck, state that mercury is the best remedy for framboesia.'

Wallbridge and Daniels say: 'Mercury now, as in the early times, holds the first place,' although its risks are clearly pointed out; they note how, in the West Indies, Nicholls found that 41 per cent. of medical men used mercury for the treatment of yaws, as against 26 per cent. who used arsenic. They continue: 'Both potassium iodide and mercury in many cases cause speedy disappearance of the eruption, in fact, often more markedly than in the case of syphilis.' The same observations about the efficacy of mercury in yaws have been made from the early times, when slaves were treated with mercury in order to conceal the disease when they were exposed for sale. Hutchinson says that this divergence of opinion is merely a repetition of the experience of mercury in syphilis. For mercury to be effective in syphilis the method of use is all important; it

should be 'begun early, given in very small doses, salivation avoided, and the course continued for a year.'

It appears possible that a source of fallacy has recently been introduced into this question, the fallacy being to compare the results of mercury-plus-arsenicals treatment as used to-day for syphilis, with the results of mercury alone as used in the old days for yaws.

That syphilis responds well to mercury requires decided qualification. Thus Harrison emphasized how extremely unsatisfactory the early mercurial treatment of syphilis proved, as compared with the later treatment of syphilis, in which mercury was combined with arsenicals. 'Under systematic mercurial treatment, 83 per cent. of soldiers suffering from syphilis required readmission to hospital at least once during the first year for reappearance of contagious lesions. In contrast with this, the readmission for clinical relapse amongst over ten thousand cases of syphilis treated with "606" and mercury, whose records are accessible to the writer, have been less than 1.3 per cent. Under mercurial treatment a soldier spent an average of 66.2 days in hospital during the first year of the disease, while the average time spent by soldiers under "606" and mercurial treatment at Rochester Row at the time these notes were collected was twenty-five days.'

It appears from the above evidence that the differential tabulation acts in two ways: it makes a serious understatement of the value of mercury in yaws, and a correspondingly large exaggeration of the value of mercury in syphilis; these under- and over-statements cancel each other and thereby vitiate fundamentally this therapeutic criterion, and appear to render it valueless for purposes of differentiation.

In concluding this analysis, attention may be drawn to some misconceptions which rigid tabulations of this kind tend to inculcate, and to certain omissions which seriously prejudice the student, in his approach to the problem. The idea is given that syphilis is a single clinical entity, suitable as a standard of comparison; yet syphilis, even in temperate climates, is by no means a uniform disease which will serve as a simple or suitable basis of comparison with any other disease; the venereally-acquired, the infantile-acquired, and the congenital each deserve to be considered separately.

Again, the numerous and very real differences which exist clinically between even venereally acquired syphilis among natives of the tropics and the similar disease among inhabitants of temperate regions are either entirely overlooked, or at any rate not considered worthy of mention. Yet the clue to the solution of the yaws and syphilis question appears to lie in syphilis as it occurs in the natives of the tropics. The observations of Harrison and Lacapère, already mentioned, prove beyond all reasonable doubt, that the syphilis of natives of the tropics is no other than the syphilis of Europe, although its clinical manifestations among natives are often markedly different. The effects of extragenitally acquired syphilis in the native children of rural areas of the tropics are those about which we chiefly require information.

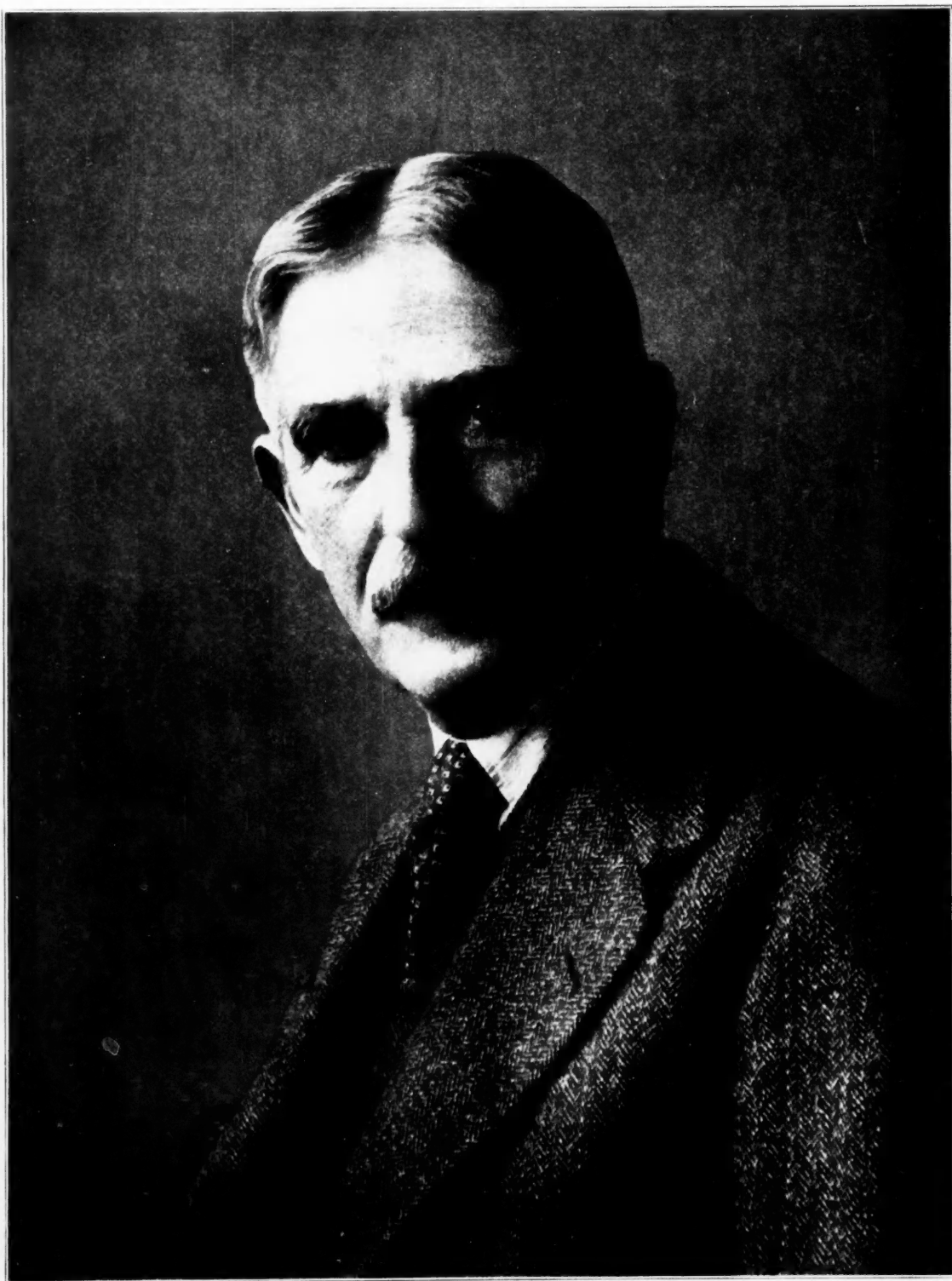
SUMMARY AND CONCLUSION

1. An attempt has been made to approach the yaws and syphilis controversy without prejudice, a notoriously difficult matter.
2. The usually accepted differential criteria have been discussed and examined from various aspects.
3. It is shown that in compiling the differential tables, fallacies have been introduced chiefly owing to comparisons being made between things which are not properly comparable.
4. Arguments are brought forward against comparing yaws, which is mainly a non-venereal disease of children in rural areas of the tropics and which begins by an extragenital primary lesion, with venereal adult syphilis of Europeans.
5. The accounts of adult venereal syphilis as it affects natives in tropical countries, are not as yet complete, but so far as they go they indicate that such syphilis varies as greatly, in many respects, from venereal syphilis in adults in temperate climates, as does yaws.
6. The evidence that yaws is other than syphilis modified by age, race and various local conditions, does not appear convincing; the onus of proof rests on those who maintain that these are two distinct diseases.

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Portrait by M. Brown

L. Fridalen ass Hunt.

IN MEMORIAM

The Editors regret to announce the death
on the 26th October, 1932, of DR. JOHN
MIDDLEMASS HUNT, Hon. Dean of the School
since July, 1921.

THE GENUS *TRICHOSTRONGYLUS*

Looss, 1905

BY

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(From the Parasitological Department in the Liverpool School of Tropical Medicine)

(Received for publication 28 March, 1931)

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INTRODUCTION

The genus *Trichostrongylus* is an important genus as it parasitises both man and domestic and other animals with pathological effects in most cases. The literature regarding it is scattered throughout different annals, circulars and magazines (published in various languages), many of which are difficult to obtain owing to their rarity. Many of the species have previously been too inadequately described and figured for up-to-date requirements; moreover, some of the species required verification.

The author, in revising this genus, has collected the literature, examined and re-described all the species, and re-figured them, with the exception of *T. probolurus* and *T. orientalis*, which definitely belong to the genus, and *T. fiberius* and *Strongylus pigmentatus*, which are not sufficiently known and may belong to the genus; unfortunately, the writer was unable to get material of these species for examination.

T. extenuatus was found to be identical with *T. axei*, and *T. delicatus* identical with *T. colubriformis*.

New hosts and localities are recorded, *T. colubriformis* was recorded from two new hosts, i.e., the sable antelope (*Hippotragus niger*) and the squirrel, *Sciurus aberti mimus*. *T. capricola* is recorded for the first time in England and *T. probolurus* for the first time in India (Punjab).

The genus *Libyostrogylus* Lane, 1923, which is considered by some authors (Baylis and Daubney, 1926) to be a synonym of the genus *Trichostrongylus*, is found to be a distinct genus for reasons to be discussed later in detail. *T. asymmetricus* Cameron, 1926, is taken out of the genus* to be put together with two new species in a separate new genus; the reasons for this change will be discussed later in detail.

Strongylus nodulosus (Rud., 1803), a parasite of the gizzard of the goose (*Anser domestica*) and *Strongylus quadriradiatus* (Stevenson, 1904), from the intestine of pigeons (*Columba* sp.), which were included by Shipley (1909) in the genus *Trichostrongylus*, are quite different from the type and are placed now respectively in the genera *Amidostomum* Railliet and Henry, 1909, and *Ornithostrogylus* Travassos, 1914. *Ostertagia callis* (Travassos, 1914) in *Didelphys aurita*, which was considered by Travassos as a member of the genus *Trichostrongylus*, in fact belongs to the genus *Ostertagia*.

Nematodirus filicollis (Rudolphi, 1802), in sheep, goats, cattle, etc., considered as a member of the genus in the *Index-Catalogue of Medical and Veterinary Zoology*, by Stiles and Hassall, belongs to the genus *Nematodirus*.

ACKNOWLEDGMENTS

The writer is greatly indebted to Professor D. B. Blacklock, M.D., of the Liverpool School of Tropical Medicine, for the kind help and many facilities for work given by him; to Dr. H. A. Baylis, of the British Museum (Natural History), London, for the accommodation and facilities for examining some of the material in his department in the British Museum; to Dr. M. Hall, Chief of the Zoology Department of the Bureau of Animal Industry, Washington, D.C., for lending for examination some of the material belonging to the genus; to Mr. A. W. N. Pillers, F.R.C.V.S., of Liverpool, for lending some of the material to be examined; to Colonel W. A. Wood, M.R.C.V.S., of Cambridge, for allowing the writer to examine his material of the two new species and for lending his unpublished manuscript; and to Mr. R. H. Burne, F.R.S., of the Royal College of Surgeons, London, for accommodation and facilities and for permission to examine Cobbold's types of *Libyostrogylus douglassii* and Lane's types of *L. hebreunicutus*.

* *T. australis* and *T. dissimilis* described by Wood from the Wallaby (*Macropus woodwardi*). n.g. *Asymmetricostrongylus*.

THE GENUS *TRICHOSTRONGYLUS*

AND ITS CLASSIFICATION

Order STRONGYLOIDEA Weinland, 1858.

Males with a terminal or subterminal caudal bursa, supported by a system of rays consisting typically of six paired main rays and a median unpaired dorsal ray with accessory branches. Oesophagus more or less club-shaped, without a posterior bulb.

Family TRICHOSTRONGYLIDAE Leiper, 1912.

Body more or less filiform, mouth simple, directed straight forward; buccal capsule usually absent or rudimentary, occasionally relatively well developed, without anterior cutting organs or corona radiata. Bursa copulatrix with well-developed lateral lobes, dorsal lobe either not differentiated or very small, near the cloaca is often to be found a supporting structure of variable form known as the telamen. Parasitises of the alimentary canal of vertebrates.

Sub-family *Trichostrongylinae* Leiper, 1908.

More or less filiform worms; buccal capsule rudimentary or absent. Spicules either long and filiform, or short and stout, with crests and protuberances. Female with double genitalia.

Genus *Trichostrongylus* Looss, 1905.

Cuticle of the anterior end may be raised into swellings. Cervical papillae absent. Buccal cavity ill defined. Bursa with relatively large lateral lobes but without well-developed dorsal lobe. Ventral rays widely separated and of very different thicknesses; the ventro-ventral thin and ventrally directed, the latero-ventral thick, divergent from the ventro-ventral and close to the lateral rays. Postero-lateral ray thinner than, and divergent from, the remaining lateral rays, lying close to the externo-dorsal ray. Dorsal ray cleft near its tip, ending in short digitations. Pre-bursal papillae small or absent. Spicules short, spoon- or spatula-shaped, appearing twisted on account of ridges on their surfaces. An accessory piece present. Vulva typically with protruding lips. Eggs thin-shelled, segmenting when deposited. Parasites in the alimentary tract of herbivores, rodents, birds and man. Type species, *T. retortæformis* (Zeder, 1800).

LIST OF SPECIES

- T. retortæformis* (Zeder, 1800) Looss, 1905.
T. colubriiformis (Giles, 1892) Ransom, 1911.
T. capricola Ransom, 1907.
T. axei (Cobbold, 1879) Railliet and Henry, 1909.
T. vitrinus Looss, 1905.
T. falcatus Ransom, 1911.
T. rugatus Monnig, 1925.
T. tenuis (Mehlis, 1846) Shipley, 1909.
T. pergracilis (Cobbold, 1873) Shipley, 1909.
T. affinis Graybill, 1924.
T. calcaratus Ransom, 1911.
T. probolurus (Railliet, 1896) Looss, 1905.
T. orientalis Jimbo, 1914.

SPECIES NOT SUFFICIENTLY KNOWN WHICH MAY BELONG TO THE GENUS *TRICHOSTRONGYLUS*

- T. fiberius* Barker and Noyes, 1915.
Strongylus pigmentatus von Linstow, 1904.

DESCRIPTION OF SPECIES

Trichostrongylus retortæformis (Zeder, 1800) Looss, 1905.

About fifty worms of this species were examined. The bulk of the material from a hare in Suffolk, England, was kindly lent by A. W. N. Pillers, Esq., F.R.C.V.S. The rest was in the museum of the Liverpool School of Tropical Medicine and was collected from: (a) the small intestine of a mountain hare, Scotland; (b) rabbit, locality unknown.

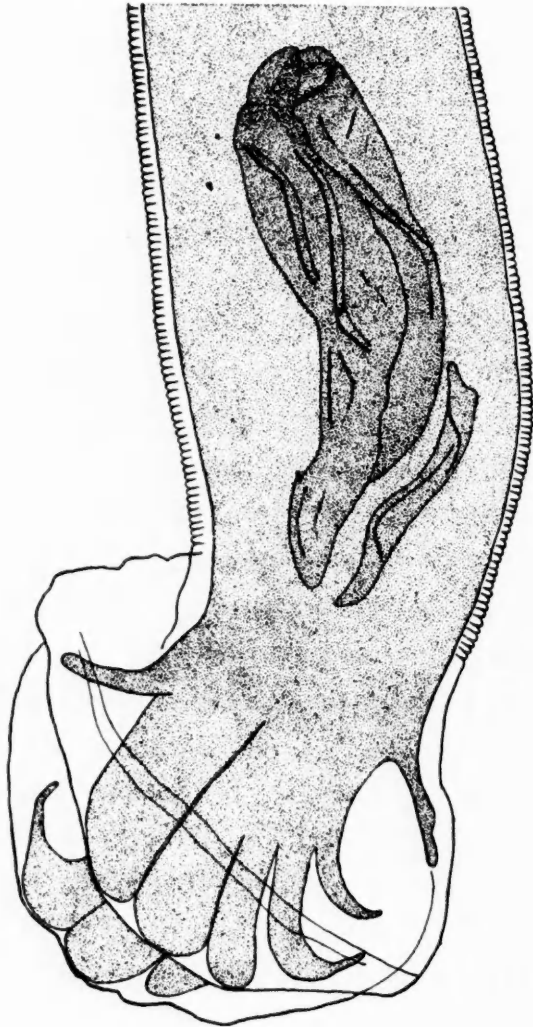
The worms are small and very slender. The body is gradually attenuated anterior to the genital opening. The head is provided with three inconspicuous lips and small punctiform papillae. Buccal cavity weakly developed. There are no cervical papillae. The head measures from 10μ to 18μ in width and the cuticle surrounding it is often inflated. Excretory pore is from 82μ to 154μ from the head end and lies in a well-marked depression in the cuticle. The cuticle is transversely striated. The oesophagus is long and simple, measuring from 657μ to 874μ ; only in one case of a female was it more than 1 mm. and measured 1.066 mm.

The male measures 5.349 mm. to 7.2 mm. in length, and 72μ to 110μ in maximum breadth just anterior to the bursa. The testis is not coiled. The bursa has two fairly large lateral lobes in comparison with the size of the worm; each lobe is supported by six rays. The distal free ends of the lobes are inwardly curled in all the material examined and several attempts to spread them were unsuccessful. They measure about 130μ dorso-ventrally. The dorsal lobe is indistinguishable from the lateral lobes. The ventro-ventral ray is thin and directed ventrally and almost reaches the edge of the bursa, which is indented opposite the tip of the ventro-ventral ray. The latero-ventral ray is widely separated from the ventro-ventral; it is quite thick and is close to the laterals. The latero-ventral with the laterals form a series of rays decreasing gradually in thickness dorsally. The tip of the externo-dorsal ray is about half-way between the tips of the dorsal and postero-lateral. The postero-lateral ray ends relatively far from the margin of the bursa and its extremity is slightly bent towards the dorsal ray. The externo-dorsal arises in common with the dorsal; both rays are thicker at their proximal than at their distal parts. The dorsal ray almost reaches the margin of the bursa, measuring from 35μ to 43μ , and bifurcates at about one-fifth of the whole length of the ray; each of these bifurcations is again divided into two very thin and pointed divisions.

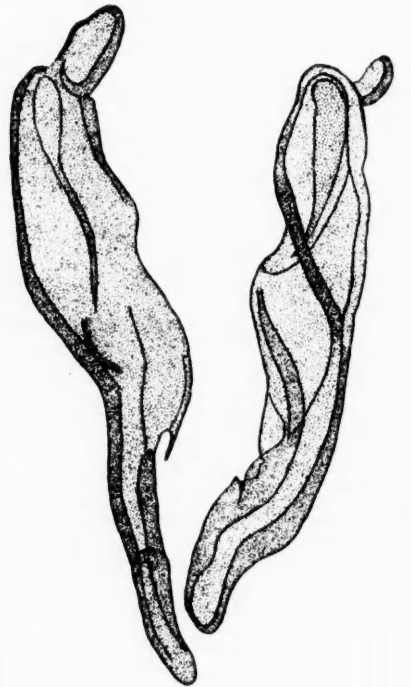
The spicules are dark brown in colour, are unequal, the left being slightly longer than the right, and of different shapes. Both spicules are bent longitudinally, the convexity is dorsal and the concavity ventral, the dorsal edges are thin and smooth, the proximal ends of the spicules have a disc-like process, the plane of this process in the case of the left spicule is directed antero-posteriorly, in the case of the right spicule transversely. The spicules are bluntly pointed at their distal ends and bear ventrally small hook-like processes which are directed anteriorly and appear on lateral view, and are situated at a distance of about 36μ from the posterior end; that of the right spicule is more blunt than that of the left. *The left spicule* measures from 132μ to 158μ in length and shows laterally two thick longitudinal ridges on its right side which give the impression that it is folded towards this side (these ridges being merely the edges of the folds). It has on its inner side a sharp backwardly projecting



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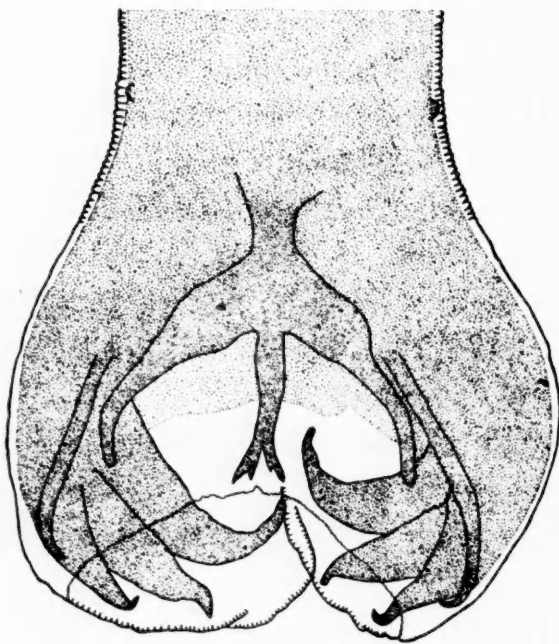
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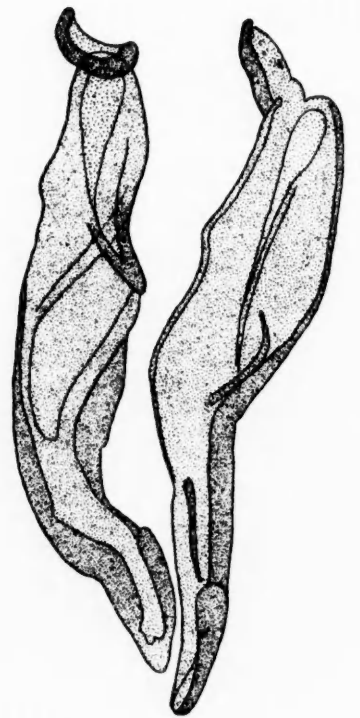
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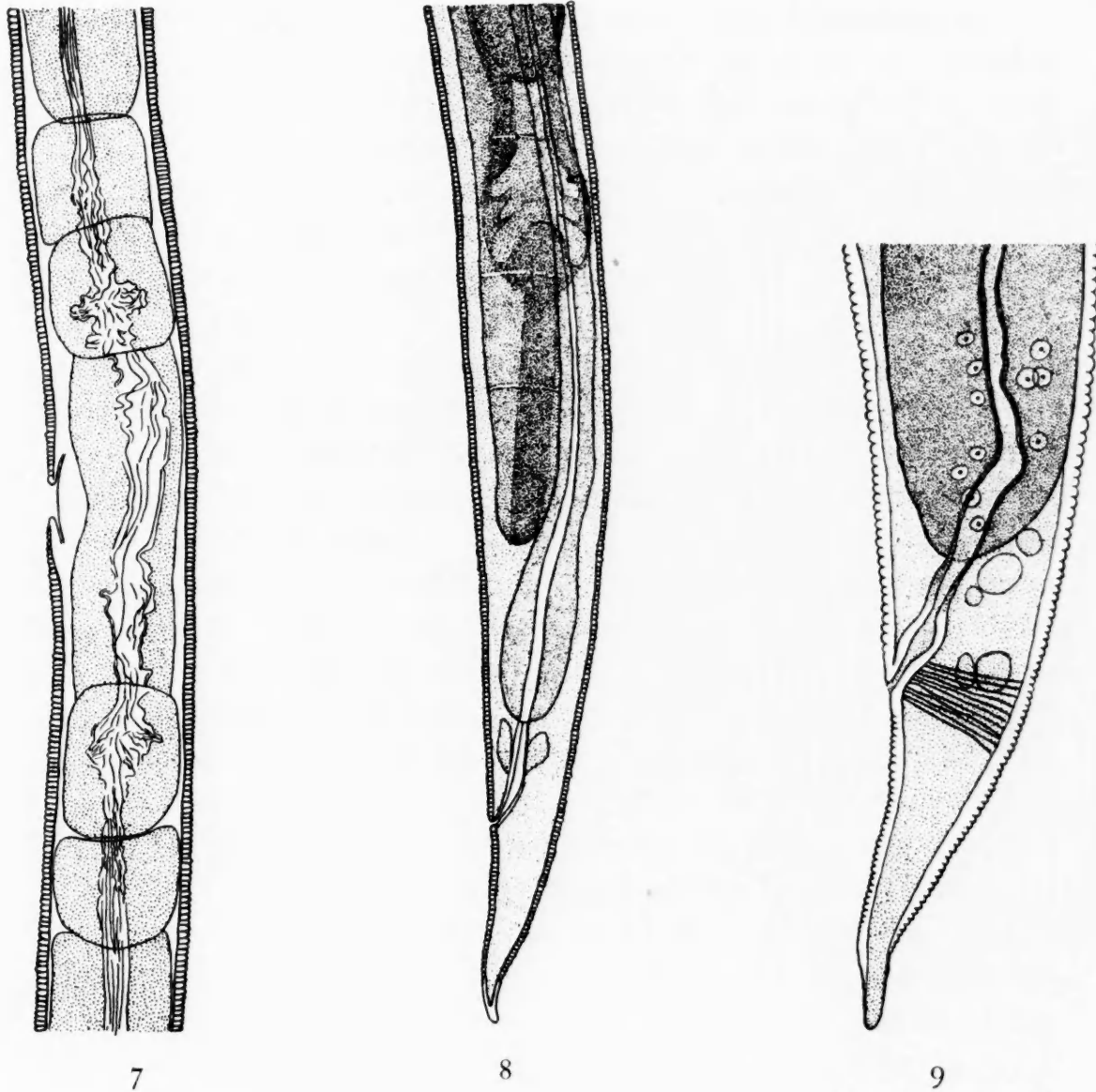
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FIGS. 1 to 9. *T. retortaeformis* 1.—Head end, lateral view, note excretory pore on the left side of the figure. $\times 400$. 2.—Head end of a female to show the cuticle inflated at times. $\times 400$. 3.—Bursa, spicules and gubernaculum; left lateral side. $\times 400$. 4.—Bursa, dorsal view. $\times 400$. 5.—Spicules, dorsal view. $\times 450$. 6.—Spicules, ventral view. $\times 450$. 7.—Region of the vulva and ovejectors of a female worm. $\times 247$. 8.—Posterior end of a female worm. $\times 247$. 9.—Posterior end of a female worm. $\times 400$.

process slightly in front of the junction of the posterior and middle thirds. This process is very thin and transparent and thus is easily overlooked. The right spicule measures from 115μ to 144μ and bears on its ventral surface a blunt knob-like protuberance at the junction of the anterior and middle thirds; it has a collar-like elevation at the anterior third, the narrow posterior end of which elevation is twisted towards the left side; behind the collar there are two prominent parallel elevations which give the impression that the right spicule is spirally twisted.

The gubernaculum measures from 40μ to 79μ in length and is not chitinised to the same extent as the spicules. Viewed from the dorsal or the ventral surfaces it is boat-shaped with narrow elongated anterior and posterior portions; the anterior is longer.

The female measures from 5.352 mm. to 8.1 mm. in length and from 66μ to 108μ in maximum breadth at the region of the vulva. The latter is a longitudinal slit slightly crescentic with definite cuticular lips, measures 66μ to 74μ in length and is from 1.17 mm. to 1.8 mm. from the tip of the tail. The uteri are divergent and there are well-developed ovejectors, the combined length of which is from 374μ to 540μ . The anterior ovary bends backwards at about 1.8 mm. from the head end and the posterior one bends forwards at about 270μ from the tip of the tail. The posterior end of the worm gradually narrows from the point where the posterior ovary bends forwards. The anus is situated at a distance of from 82μ to 123μ from the tip of the tail. The tail is usually curved ventrally and in some specimens the tip curves dorsally. The diameter of the body at the region of the anus is from 28μ to 39μ ; the latter is but slightly raised from the surface of the body.

Intrauterine eggs are thin-shelled, elongated and relatively large, measuring from 79μ to 96μ long by 36μ to 44μ broad.

Host and Habitat. In the small intestine (duodenum), rarely in the stomach of *Oryctolagus cuniculus* (*Lepus cuniculus*); *Lepus europaeus* and *Viscacia viscacia*.

Geographical distribution. Europe and the United States of America.

Hall, in his book 'Nematode Parasites of Mammals of the Orders Rodentia, Lagomorpha and Hyracoidea,' page 125, states that 'The anus is only slightly salient and is 1 to 1.2 mm. from the tip of the tail.' This is evidently a misprint because, in the description of Looss from which Hall's description was taken, it is stated that 'Entfernung zwischen After und Schwanzspitze, 0.1-0.12 mm.'

This parasite was recorded, for the first time in Holland, by E. A. R. Baudet (1928), in a hare, and was said to cause emaciation. It was also reported by Gedoelst, 1911, in the goat, ox, sheep, but this lacks confirmation.

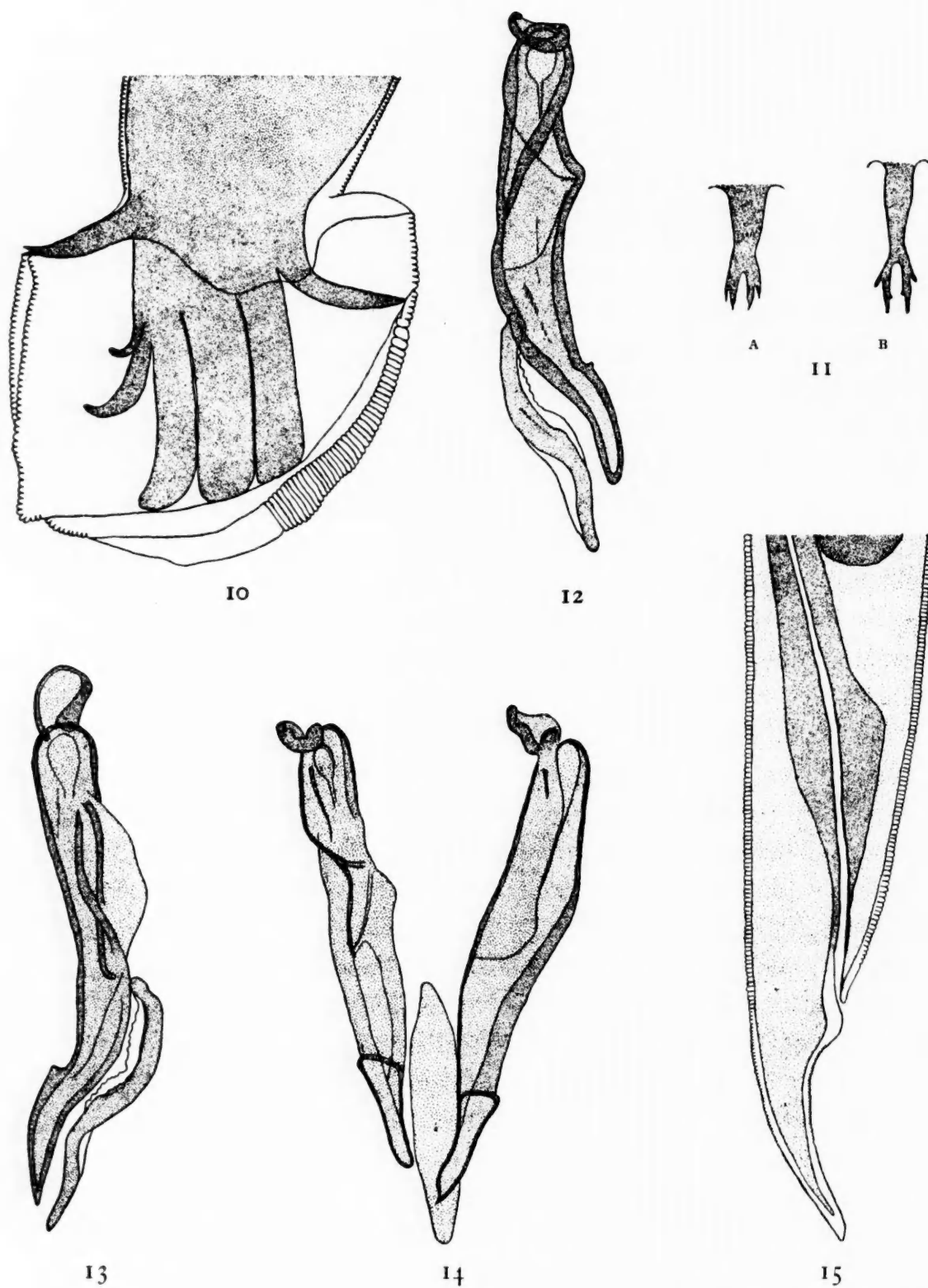
According to Railliet this parasite coexists with *Graphidium strigosum* (Dujardin, 1845) Railliet and Henry, 1909, and helps to give rise to a pernicious anaemia in rabbits.

Trichostrongylus colubriiformis (Giles, 1892) Ransom, 1911.

SYNONYMS: *Strongylus colubriiformis* Giles, 1892.
Strongylus instabilis Railliet, 1893.
Strongylus subtilis Looss, 1895.
Strongylus retortæformis Zeder, 1800, pro parte?
Trichostrongylus instabilis (Railliet, 1893) Looss, 1905.
Trichostrongylus subtilis Looss, 1905.
Trichostrongylus delicatus Hall, 1916.

Historical. Giles, in 1892, in Shillong (Assam) and Sanawar (Punjab), British India, during investigations on the nodular disease of sheep due to *Oesophagostomum columbianum*, found a nematode in the small intestine which he described and named as *Strongylus colubriiformis*. Railliet, in 1893, found a strongyle in small quantities in the abomasum and in large quantities in the small intestine, especially in the duodenum of sheep, goat and deer, causing pernicious anaemia and death; this worm he described and named *Strongylus instabilis*. He also pointed out the great similarity to, and even the possible identity of his species with the *Strongylus colubriiformis* described by Giles a year before.

In 1895 Looss described a strongyle from the small intestine (first 50 cm. from the pylorus) of man in Egypt, which he called *Strongylus subtilis*. He later (1905) introduced the genus *Trichostrongylus* to include these and other allied species and after examining Railliet's material of *T. instabilis* he was satisfied that *T. subtilis* Looss, 1905, was identical with *T. instabilis* (Railliet, 1893) Looss, 1905. Thus the name *T. subtilis* has fallen out as a synonym according to the rules of Zoological nomenclature, and the question then arose whether *T. instabilis* was identical with *T. colubriiformis*, as the author of the former worm himself (Railliet) has thrown doubt on the matter as has also (later) Ransom, in his book 'Nematodes Parasitic in the Alimentary Tract of Ruminants' (1911). This question was satisfactorily settled by Clayton Lane, in a paper published in 1913, in which he proved that *T. instabilis* is identical with *T. colubriiformis* by examining Giles's original material and obtaining specimens himself from sheep from nearly the same locality from which Giles obtained his material. Thus the name *T. colubriiformis* (Giles, 1892) Looss, 1905, stands as it has the priority over the others.



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FIGS. 10 TO 15. *T. colubriformis*. 10.—Bursa, right lateral view. $\times 400$. 11 (A and B).—Dorsal rays. $\times 400$. 12.—Gubernaculum and right spicule, right lateral view. $\times 450$. 13.—Gubernaculum and left spicule, left lateral view. $\times 450$. 14.—Spicules and gubernaculum, ventral view. $\times 450$. 15.—Posterior end of a female. $\times 400$.

In 1916, Hall described a new species which he called *Trichostrongylus delicatus* from the small intestine of the squirrel, *Sciurus aberti mimus*, from Pagosa Springs, Colorado. On examination of co-types of this species kindly lent for examination by Dr. M. Hall, the writer of the present paper found that it was identical with *T. colubriformis* (Giles, 1892). The writer communicated with Dr. Hall for a confirmation of the finding. The following extract is from the letter dated February 28th, 1931, of the writer of the present paper, to Dr. Hall :

'With regard to the material labelled *T. delicatus* Hall 1916, you have sent one male and one female which, on examination, I found to be *T. colubriformis* (Giles, 1892) Ransom, 1911 = *T. instabilis* (Railliet, 1893) Looss, 1905, and *T. subtilis* Looss, 1905. I have made six figures with the aid of a camera lucida of this species, *T. delicatus*, which I enclose herewith for comparison with the literature. The shape of the spicules and gubernaculum, the dorsal ray and the shape of the other rays and the posterior extremity of the female are convincing, as are also the measurements of the different parts.'

In answer, Dr. Hall agrees to my finding, saying :

'I have your letter of February 28th, and the figures of *Trichostrongylus delicatus*, and have turned these over to Mr. B. G. Chitwood, of this division, for a check. On comparing *T. delicatus* with *T. colubriformis*, he concurs in your finding to the effect that the two species are identical. Apparently there is nothing for it but to sink *T. delicatus* as another synonym.'

The material available for examination was as follows :—

1. From sable antelope (*Hippotragus niger*) Zoo, Calcutta.
This is apparently a new host to be recorded to contain the parasite. Eleven worms were available for examination, four of which were males.
2. From the small intestine of lamb (*Ovis aries*), England.
Five worms were available for examination, two of which were males.
3. From the fourth stomach of a Maltese goat (*Capra hircus*) from Bethesda, Maryland, U.S.A., came under cover of material labelled *T. capricola* ; co-types kindly lent by Dr. M. Hall. One male worm was available for examination.
4. From the small intestine of a goat from Bromsgrove, England. The material was a mixed one of *T. colubriformis*, *T. capricola* and *T. axei*. There was a great number of the first two worms available for examination.
5. From sheep, South Africa. A mixed infection of *T. colubriformis* and *T. rugatus*. A large number of worms was available.
6. From sheep from the Punjab. Giles's type material ; examined in the British Museum (Natural History).

7. Co-types of *T. instabilis* from the small intestine of *Ovis aries*, Buenos Ayres, from the collection of Raillet kept in Cairo.
8. From *Camelus dromedarius*, Alexandria.
9. From the duodenum and jejunum of *Homo sapiens*; this is the type material of Looss' *T. subtilis* from man in Cairo.
10. From the intestine of *Cercopithecus patas*, Egypt.

Forty-five worms of *T. colubriiformis* were thoroughly examined and measured, eleven of which were females. This is no ratio as the writer purposely examined more males than females.

The worms are small and very slender, reddish or whitish in colour. The body is gradually attenuated anterior to the genital opening. Buccal cavity weakly developed. The head is provided with three inconspicuous lips and punctiform papillae. There are no cervical papillae. The head measures from 13μ to 17μ in width. The excretory pore is situated at a distance of 114μ to 171μ from the cephalic end, and lies in a depression in the cuticle. The cuticle is transversely striated. The oesophagus is simple, measuring from 644μ to 915μ , and in one case only in a female worm 1.021 mm.

The male measures 4.389 mm. to 7.714 mm. in length and 66μ to 110μ in maximum breadth in front of the bursa. It has a rather large bursa with the dorsal lobe very weakly developed. The lateral lobes as in the former species (*T. retortæformis*) are always curled inwards. The bursal formula is as follows:—Externo-lateral ray usually the broadest, but is sometimes of the same width as the latero-ventral and medio-lateral, which as a rule are narrower. Postero-lateral ray small in comparison to the other laterals; its tip is directed towards the externo-dorsal ray, the distance between the tips of the postero-lateral and externo-dorsal rays—which are on the same line antero-posteriorly—is shorter than the distance between the tips of the externo-dorsal and the dorsal. The dorsal ray measures from 39μ to 52μ in length and at about its posterior third it bifurcates; the inner divisions are longer than the outer and bear on their inner surfaces a small papilla-like projection which is more marked in some than in others. The main stem of the ray gradually narrows from its proximal end till the point of bifurcation.

The spicules are dark brown in colour and are unequal, the left spicule being longer and broader than the right. As a very characteristic thing each spicule possesses a well-defined sharp hook-like

process which is developed on the ventral side at about one-quarter of the length of the spicule from the posterior end and although these are small they are very distinct and can be seen best from a side view of the spicule. Both spicules are bent ventrally. *The left spicule* measures from 136μ (hook 35μ from posterior end) to 171μ (hook 39μ from posterior end), its posterior tip is more sharply pointed than that of the right spicule. *The right spicule* is shorter than the left and measures from 123μ (hook 30μ from posterior end) to 154μ (hook 41μ from posterior end), it bears ventrally a rounded protuberance at about the junction of the anterior and middle thirds of the spicule. The posterior end of the spicule is rounded. The collar-like thickening described in the right spicule of *T. retortæformis* is also seen here. It is interesting to notice from the accompanying tables that although the lengths of the left and right spicule are always different in the one and the same worm, the distance of the hook from the posterior end of the spicule is nearly always the same in the two spicules of the same worm.

The gubernaculum measures 66μ to 88μ in length and is boat-shaped when viewed dorsally or ventrally; viewed laterally it appears to be composed of a narrow chitinised band which comprises two and a half curves, the concavity of the first curve, which is the largest, is directed ventrally, that of the second curve dorsally, and that of the half curve again ventrally. There is a very slightly chitinised piece with an undulated free edge filling the first concavity and a similar one which has no undulated free edge filling the second concavity. In some cases the anterior end of the gubernaculum is so curved as to resemble the handle of a walking stick viewed from the side or sometimes it ends in a small knob.

The female measures from 5.092 mm. to 8.626 mm. in length, and from 79μ to 118μ in maximum breadth in the region of the vulva. The latter is a longitudinal slit, crescentic in shape, and the cuticle in this region is almost always raised considerably from the surface. The slit measures from 39μ to 61μ in length and is situated at from 1.007 mm. to 1.786 mm. from the tip of the tail. The uteri are divergent and there are well-developed ovejectors, the combined lengths of which are from 396μ to 624μ , but, as is shown in the accompanying table, the length of ovejectors in this species is usually over 400μ . The anterior ovary bends backwards at from 1.034 mm. to 2.014 mm. from the cephalic end and the posterior one bends

forwards at from 176μ to 387μ from the tip of the tail. The anus is situated at from 66μ to 92μ from the caudal extremity. The diameter of the body at the region of the anus is from 26μ to 48μ . The body gradually but slightly tapers from the loop of the posterior ovary to the anus and then suddenly to the tip of the tail which is sharply pointed.

Intrauterine eggs are thin-shelled, large and elongated, with the ovum inside in the morula stage; they measure from 79μ to 96μ long by 35μ to 48μ broad.

Hosts infected. In the duodenum and stomach of man (*Homo sapiens*); Arabian baboon (*Papio hamadryas*); chimpanzee (*Anthropopithecus troglodytes*); rhesus monkey (*Macacus rhesus*); pig-tailed macaque (*Macacus nemestrinus*); Siamese macaque (*Cynomolgus umbrosus*); Chinese macaque (*Cynomolgus sinicus*); squirrel (*Sciurus aberti mimus*); and in the duodenum and fourth stomach of sheep (*Ovis aries*); goat (*Capra hircus*); Arabian camel (*Camelus dromedarius*); Bactrian camel (*Camelus bactrianus*); Himalayan bharal (*Pseudois nahura*); gazelle (*Gazella dorcas*); buck (*Antilocapra americana*); sable antelope (*Hippotragus niger*).

Geographical distribution. British India, Europe, North Africa (Egypt), Armenia, United States of America and (?) Japan.

The nematodes which Ogata discovered in a Japanese woman in 1889, afterwards identified by Ijima (1895) *Strongylus subtilis*, as well as those reported by Kitamura and Oishi (1913) from South Japan and Korea, are not *T. colubriformis* (= *T. subtilis*), according to Jimbo (1914), but another species, namely *T. orientalis*.

According to Monnig, *T. colubriformis* causes heavy losses in sheep in South Africa, and according to Ransom is encountered in large numbers in animals which died following symptoms of parasitic disease in the United States of America. In man a severe secondary anaemia is produced if a large number of the worms are present.

Trichostrongylus capricola Ransom, 1907.

The material available for examination was as follows:

1. From the fourth stomach of a Maltese goat (*Capra hircus*) from Bethesda, Maryland, U.S.A. Co-types, kindly lent by Dr. M. Hall. Five worms (three of which were females) were available for examination.
2. From the small intestine of a goat from Bromsgrove, England, numerous worms. Thirty-one of these, of which four were females, were carefully examined and measured.

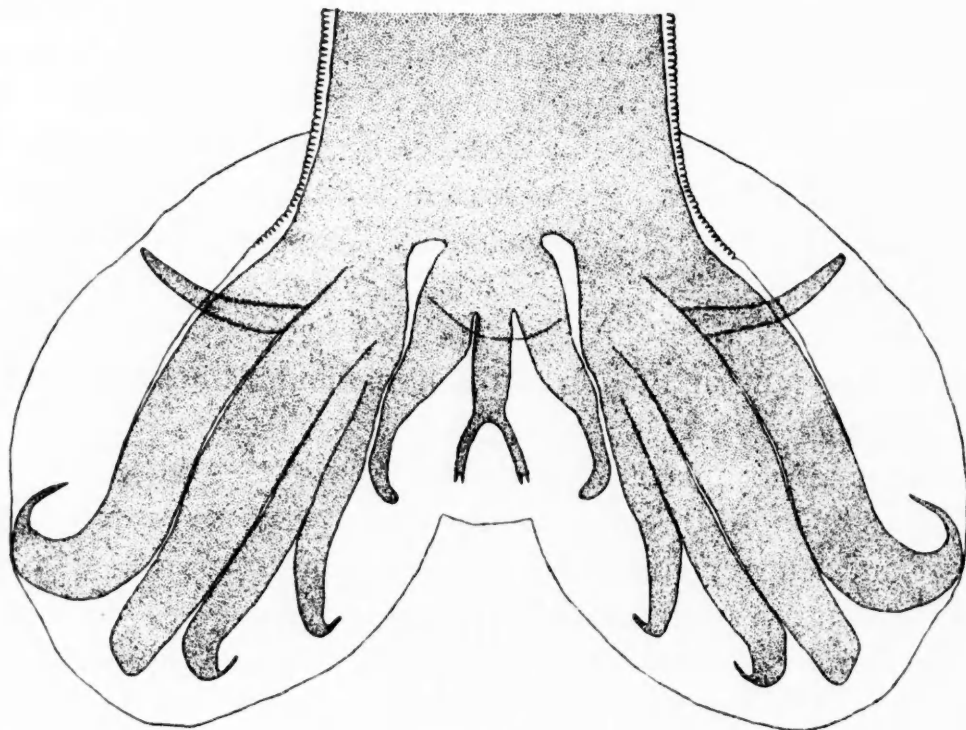
The worms are smaller in general than *T. colubriformis*. The body is gradually attenuated anterior to the genital opening. The buccal cavity is weakly developed. The head is provided with three inconspicuous lips and punctiform papillae. There are no cervical papillae. The head measures from 13μ to 17μ in width. The excretory pore is situated at a distance of 110μ to 180μ from the cephalic end and lies in a depression in the cuticle. The cuticle is transversely striated. The oesophagus is simple, measuring from 598μ to 972μ in length.

The male measures from 3.552 mm. to 5.89 mm. in length and in one case 6.042 mm. and 66μ to 110μ in maximum thickness in front of the bursa.

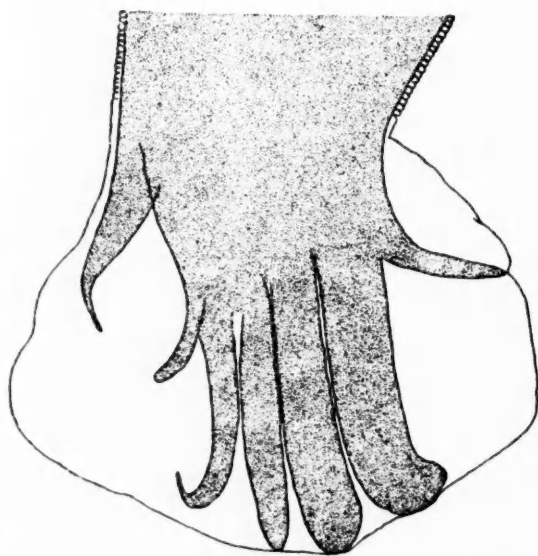
The bursal formula is as follows:—ventro-ventral ray is thin and reaches almost the edge of the bursa. Latero-ventral is the thickest and longest and is close to the laterals; towards its distal end it becomes very thin and reaches the edge of the bursa. The externo-lateral is the next in thickness, then the medio-lateral and postero-lateral. The laterals are nearly of the same length and their tips separated by equal distances. The externo-dorsal ray is short in comparison to the other rays and is very much swollen at its proximal end, but gradually narrows towards the distal end. The dorsal ray bifurcates at about its middle, the branches being nearly of the same length as the main stem or about one-third of the total length and about half as thick; each ends in two narrow, pointed tips. The whole length of the dorsal ray is 36μ (and each of the branches measures 17μ in length, i.e., a little less than half the length of the whole ray) to 48μ (and each of the branches measures 13μ). The tip of the postero-lateral is about midway between the tips of the medio-lateral and externo-dorsal.

The spicules are almost equal in length, sometimes the left is slightly longer than the right. They are yellowish brown in colour. The distal portion is narrower than the proximal and is rounded posteriorly. *The left spicule* measures 118μ to 149μ in length. *The right spicule* measures 114μ to 149μ in length. From the inner surface of both spicules arises a thin chitinated process which is directed posteriorly.

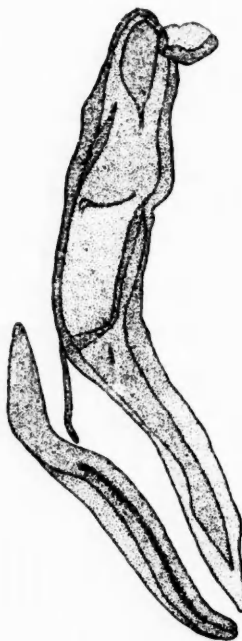
The gubernaculum measures 66μ to 88μ long. Viewed ventrally or dorsally it is spindle-shaped with the middle part very much



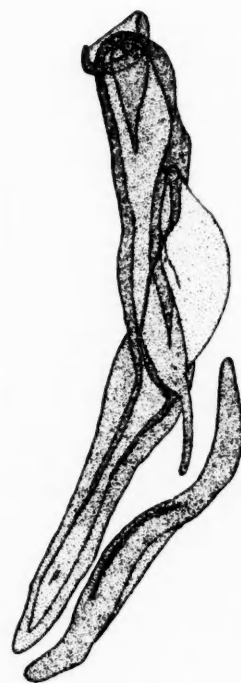
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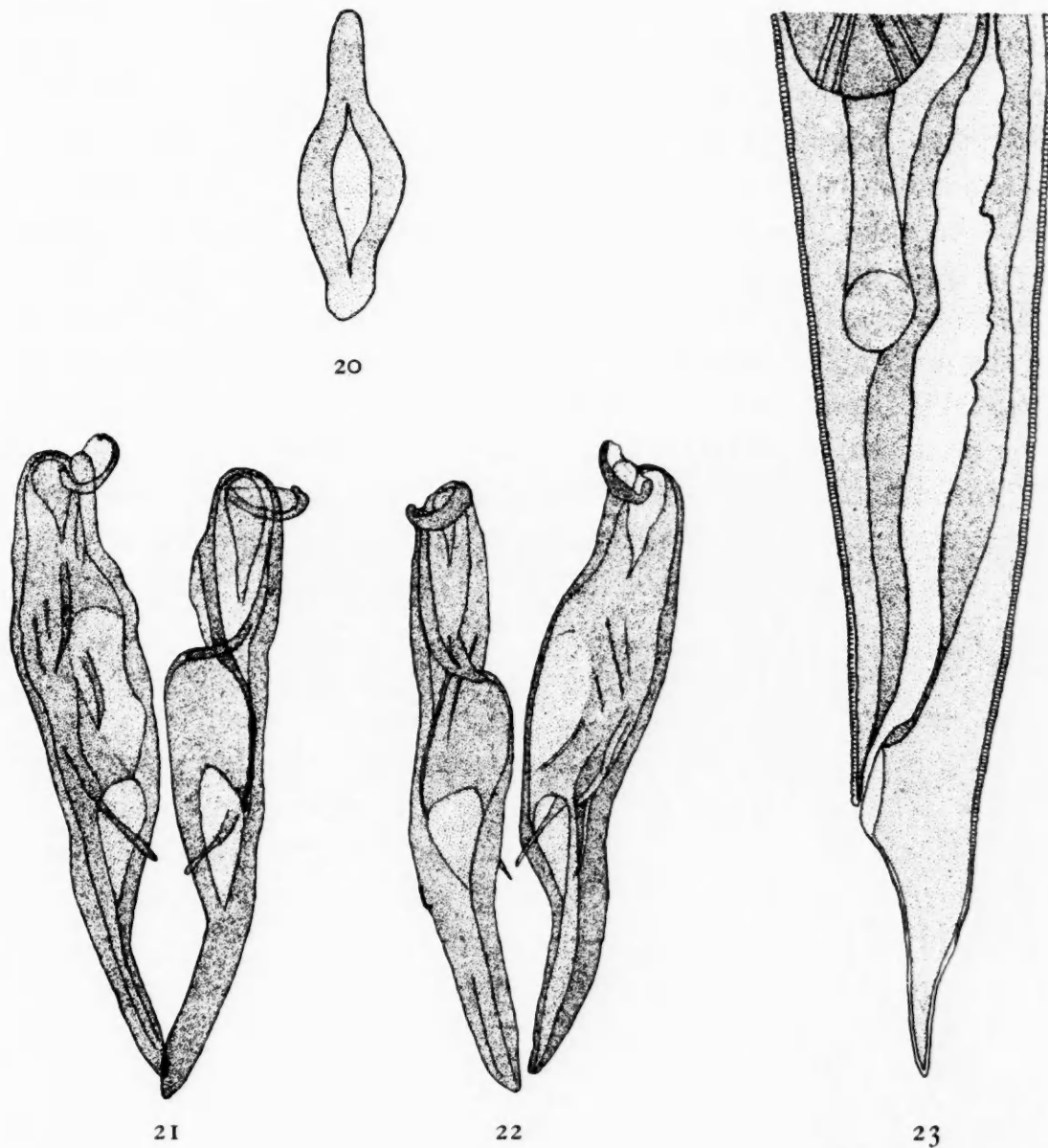
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H. F. Nagaty, *ad nat. del.*

FIGS. 16 to 23. *T. capricola*. 16.—Bursa spread, dorsal view. $\times 400$. 17.—Bursa, right lateral view. $\times 400$. 18.—Gubernaculum and right spicule, right lateral view. $\times 450$. 19.—Gubernaculum and left spicule, left lateral view. $\times 450$. 20.—Gubernaculum, dorsal view. $\times 450$. 21.—Spicules, dorsal view. $\times 450$. 22.—Spicules, ventral view. $\times 450$. 23.—Posterior end of female. $\times 450$.

swollen, the anterior part is narrow and elongated, while the posterior part is short and stout. Viewed laterally it consists of a band which resembles that described in *T. colubriformis* but much broader. The outer part of the gubernaculum is more chitinised than the middle part.

The female measures from 5.054 mm. to 6.479 mm. in length, and from 70μ to 92μ in maximum breadth in the region of the vulva.

The latter is a longitudinal slit which measures 44μ to 61μ and is situated at from 1.026 mm. to 1.235 mm. from the tip of the tail. The uteri are divergent and there are well-developed ovejectors, the combined lengths of which are from 330μ to 391μ . The anterior ovary bends backwards at from 1.094 mm. to 1.971 mm. from the cephalic end; the posterior one bends forwards at from 140μ to 250μ from the tip of the tail. The anus is situated at from 52μ to 96μ from the tip of the tail, which is sharply pointed. The diameter of the body at the region of the anus is from 30μ to 39μ . The body gradually tapers from the loop of the posterior ovary to the anus in a more marked way than in females of *T. colubriiformis*.

Intrauterine eggs are thin-shelled, large and elongated with the ovum inside in the morula stage, in one case the nearest ova to the ovejectors already developed larvae. They measure 88μ to 105μ by 39μ to 44μ .

It was rather difficult to distinguish females of *T. colubriiformis* from those of *T. capricola* in the mixed infection from the goat of Bromsgrove; often it was impossible to ascertain whether a particular female belonged to the one species or the other; in the case of males it was, of course, a very easy matter to distinguish the species, by utilizing the shape of the spicules and the bursal formula, the dorsal ray especially being an important clue; moreover, males of *T. colubriiformis* are on the whole longer than those of *T. capricola*.

The following points were observed and collectively they help to establish a differentiation between the females of the two species:—the ovejectors including the sphincters of *T. capricola* are shorter than those of *T. colubriiformis* (less than 400μ in length) and are comparatively more strongly developed; the posterior part of the female in *T. capricola* (that part behind the loop of the posterior ovary) narrows towards the anus more markedly than in *T. colubriiformis* and the tail is generally slightly longer; the lips of the vulva in *T. capricola* are not so protruding as in most individuals of *T. colubriiformis* and the size of the ova is larger than in *T. colubriiformis*.

Hosts infected. In the small intestine and fourth stomach of goat (*Capra hircus*); sheep (*Ovis aries*); prong-horned antelope (*Antilocapra americana*).

Geographical distribution. United States of America, Europe (France and England).

Trichostrongylus axei (Cobbold, 1879) Railliet and Henry, 1909.

SYNONYMS: *Strongylus axei* Cobbold, 1879.

Strongylus tenuissimus Mazzanti, 1891.

Strongylus gracilis MacFadyean, 1896 (Not *S. gracilis* Leuckart, 1842).

Strongylus extenuatus Railliet, 1898.

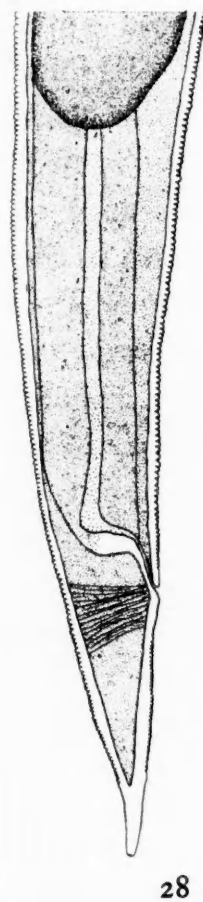
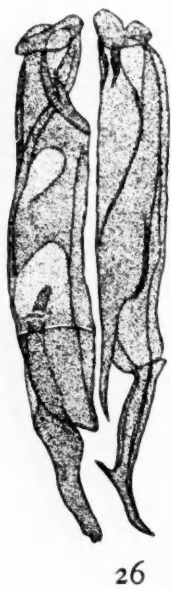
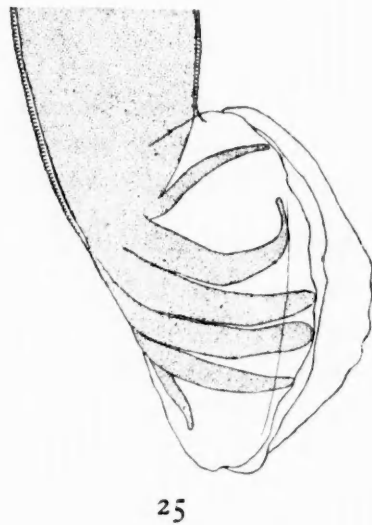
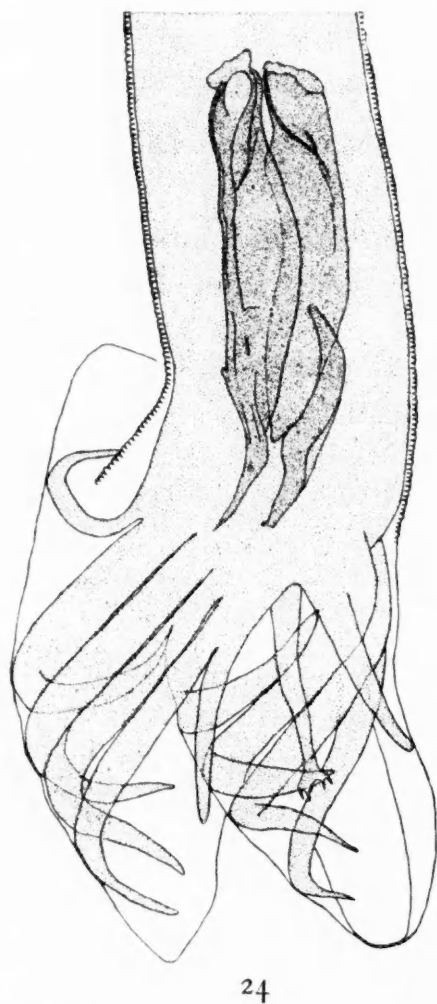
The material available for examination was as follows:—

1. From the small intestine of a goat from Bromsgrove, England.
2. From the stomach of a sheep, England.
3. From the fourth stomach of an ox.
4. From a horse, Surrey.
5. From the stomach of a horse from the Federated Malay States.

From the above-mentioned list of material a great number of worms was available for examination.

The worms are small, fine and hair-like. The body is gradually attenuated anterior to the genital opening. Buccal cavity very small. The head is provided with three inconspicuous lips and punctiform papillae. No cervical papillae present. The head measures from 8μ to 13μ in width. The excretory pore is situated at a distance of 110μ to 176μ from the head end, and lies in a depression in the cuticle. The cuticle is transversely striated. The oesophagus is simple, measuring 514μ to 668μ in length.

The male measures, in the material examined, 2.584 mm. to 3.75 mm. in length and 44μ to 66μ in maximum breadth in front of the bursa. The bursa is comparatively well developed but there is no separate dorsal lobe. The bursal formula is as follows:—The ventro-ventral ray is thin and reaches the edge of the bursal lobe. The latero-ventral ray is as thick as the externo-lateral and medio-lateral rays, or slightly thicker. The postero-lateral ray is slightly narrower than the medio-lateral ray and is longer and thicker than the same ray in other members of the genus *Trichostrongylus* in ruminants. The externo-dorsal ray is narrow and long and its tip is about midway between the tips of the dorsal ray and postero-lateral ray. The dorsal ray is comparatively long, measuring 44μ to 52μ in length and is divided at its distal end into two short divisions, each of which is again bifid; it is of about the same length as the externo-dorsal ray. The tips of the externo-lateral and medio-lateral rays are nearer together than the tips of any of the other rays.



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FIGS. 24 to 28. *T. axei*. 24.—Bursa of male, postero-lateral view. $\times 422$. 25.—Bursa of male, right lateral view. $\times 422$. 26.—Spicules and gubernaculum, ventral view. $\times 475$. 27.—Spicules and gubernaculum, dorsal view. $\times 475$. 28.—Posterior end of female. $\times 422$.

The spicules are dark brown in colour and are very dissimilar. *The left spicule* measures 96μ to 123μ in length, and is much longer than the right one. It ends posteriorly in a thin chitinised piece which looks like a long boot, of which the toes and heels are long and sharply pointed; this appearance is best seen from the ventral view. A transverse line less chitinised than the rest of the spicule separates this part already described from the rest of the spicule roughly at about the junction of the middle and posterior thirds. There is also a narrow chitinised process directed posteriorly arising on a broad base from the dorsal surface of the spicule at about its middle. *The right spicule* measures 74μ to 96μ in length. It terminates posteriorly in a blunt and rounded end and has a less chitinised line similar to that in the left spicule, also at about the junction of the middle and posterior thirds. The spicule has a narrow and sharply pointed process directed posteriorly which originates from its dorsal side.

The gubernaculum is situated dorsal to the right spicule and measures 52μ to 61μ in length, and viewed laterally it comprises a sinuous structure with three concavities, two of which are directed ventrally; these concavities are not occupied by any weakly chitinised material as in some of the other trichostrongyles. The anterior and posterior ends are blunt. Viewed ventrally or dorsally the gubernaculum is seen to be formed of a middle swollen portion and anterior and posterior narrower portions, the swollen middle portion is asymmetrical in that it is more swollen on the right side than on the left. The anterior portion is narrower and longer than the posterior and both have rounded ends. The gubernaculum is less chitinised in the centre than at the edges.

The female measures, in the material examined, from 3.268 mm. to 4.218 mm. in length and from 44μ to 61μ in maximum breadth at the region of the vulva. The latter is a longitudinal crescentic slit and on account of the opening being surrounded by thick cuticular lips it appears sausage-shaped. The slit with the lips measures 44μ to 57μ in length and is situated at from 646μ to 792μ from the tip of the tail. The uteri are divergent and the ovejectors are not so well developed as in other species. They measure 202μ to 281μ in length. The anterior ovary bends backwards at 998μ to 1.995 mm. from the head end; the posterior ovary bends forwards

at 105μ to 215μ from the tip of the tail. The anus is situated at from 61μ to 79μ from the tip of the tail. The diameter of the body at the region of the anus is from 22μ to 26μ . The body tapers gradually and regularly from the loop of the posterior ovary to the tip of the tail; the anus is situated immediately in front of a slight elevation on the surface.

Intrauterine eggs are thin-shelled, large and elongated with the embryo inside in the morula stage. They measure from 79μ to 96μ by 35μ to 48μ .

Hosts infected. In the stomach and more rarely the anterior end of the small intestine of the donkey (*Equus asinus*); horse (*Equus caballus*), and in the fourth stomach and more rarely anterior end of the small intestine of cattle (*Bos taurus*); sheep (*Ovis aries*); goat (*Capra hircus*) and several other ruminants such as bharal (*Ovis nahura*); Mexican mountain sheep (*Ovis mexicana*); prong-horned antelope (*Antilocapra americana*); mule deer (*Odocoileus hemionus*); roe deer (*Capreolus capreolus*); Newfoundland caribou (*Rangifer terrænovæ*); Axis deer (*Axis axis*); American bison (*Bison americanus*); and occasionally in man (*Homo sapiens*).

Geographical distribution. Great Britain, Europe, United States of America, and Australia.

The writer in a previous paper has shown that *T. axei* and *T. extenuatus* are identical morphologically.

After the publication of this paper the writer's attention was drawn to two papers, one by Monnig (1928) and the other by Vogelsang (1929) in which they consider that *T. extenuatus* is a synonym of *T. axei*. These two writers, however, did not give the reasons which led them to this conclusion.

Trichostrongylus vitrinus Looss, 1905.

The material available for examination was from:

- (1) the small intestine and stomach of sheep, England;
- (2) the duodenum of sheep, England. Examined in the British Museum.

The worms are fairly long and slender. The bursa in the male is rather large and well developed in comparison with other members of the genus *Trichostrongylus*. The body tapers gradually anterior to the genital opening. The head is provided with three inconspicuous lips and punctiform papillae. The buccal cavity is weakly developed. There are no cervical papillae. The head measures

13 μ in width. The excretory pore is situated 145 μ to 180 μ from the tip of the head and lies in a well-marked depression in the cuticle. The cuticle is transversely striated and is inflated at several points in the body, especially near the head and anterior to the vulva, ventrally where it sometimes forms a wavy line. The oesophagus is long and simple and measures 748 μ to 874 μ in length.

The male measures 5.605 mm. to 7.296 mm. in length and 79 μ to 101 μ in maximum breadth anteriorly to the bursa. The bursa has two lobes which in this species are better developed than in the other species of the genus. They are united dorsally and there is no distinct dorsal lobe. The bursal formula is as follows:—Externo-lateral ray is the widest, it is slightly wider than the latero-ventral which is longest and reaches the edge of the bursa. They are close together proximally but diverge distally, the former dorsally and the latter ventrally. The medio-lateral ray is narrower than the latero-ventral and like the externo-lateral does not quite reach the edge of the bursa. The tips of the externo-lateral and medio-lateral are closer together than any of the other rays. The postero-lateral ray is long and narrow and diverges nearly throughout its length from the medio-lateral; it is almost straight, nearly reaching the edge of the bursa and is of about the same length and width as the ventro-ventral ray. The externo-dorsal ray is about the same length as the dorsal and its proximal end is thickened. The dorsal ray measures 52 μ to 66 μ and is divided distally into two parts, each of which measures about 17 μ and each is divided again into two simple papilla-like processes.

The spicules are dark brown and are of the same length namely, 149 μ to 176 μ long; they end posteriorly in a sharp tapering portion without the hook-like projections found in most of the other species of the genus.

The gubernaculum measures 74 μ to 96 μ in length and is boat-shaped viewed dorsally.

The female measures 6.897 mm. to 8.132 mm. in length, and 79 μ to 96 μ in maximum diameter at the region of the vulva. The latter is a crescentic longitudinal slit measuring 44 μ to 74 μ in length and is only slightly raised from the level of the cuticle. The vulva is 1.406 mm. to 1.577 mm. from the tip of the tail. The uteri are divergent and the ovejectors are well developed; their combined length is 404 μ to 484 μ . The posterior ovary bends forwards at

480



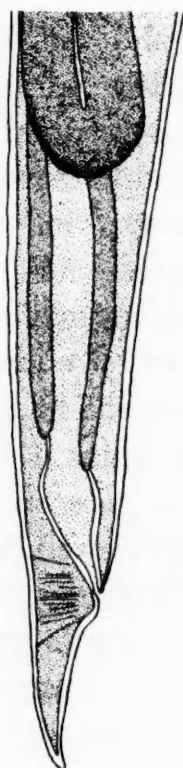
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FIGS. 29 to 32. *T. vitrinus*. 29.—Bursa, dorso-lateral view. $\times 360$. 30.—Spicules, ventral view. $\times 405$. 31.—Gubernaculum, dorsal view. $\times 405$. 32.—Posterior end of female. $\times 220$.

264 μ to 321 μ from the tip of the tail; the anterior bends backwards at 1.615 mm. to 2.47 mm. from the cephalic extremity. The posterior part of the worm narrows behind the loop of the posterior ovary as far as the anus, and then tapers rapidly to the tip of the tail. The anus is situated at a distance of 79 μ to 92 μ from the caudal extremity. The diameter of the body at the anus is 35 μ to 39 μ .

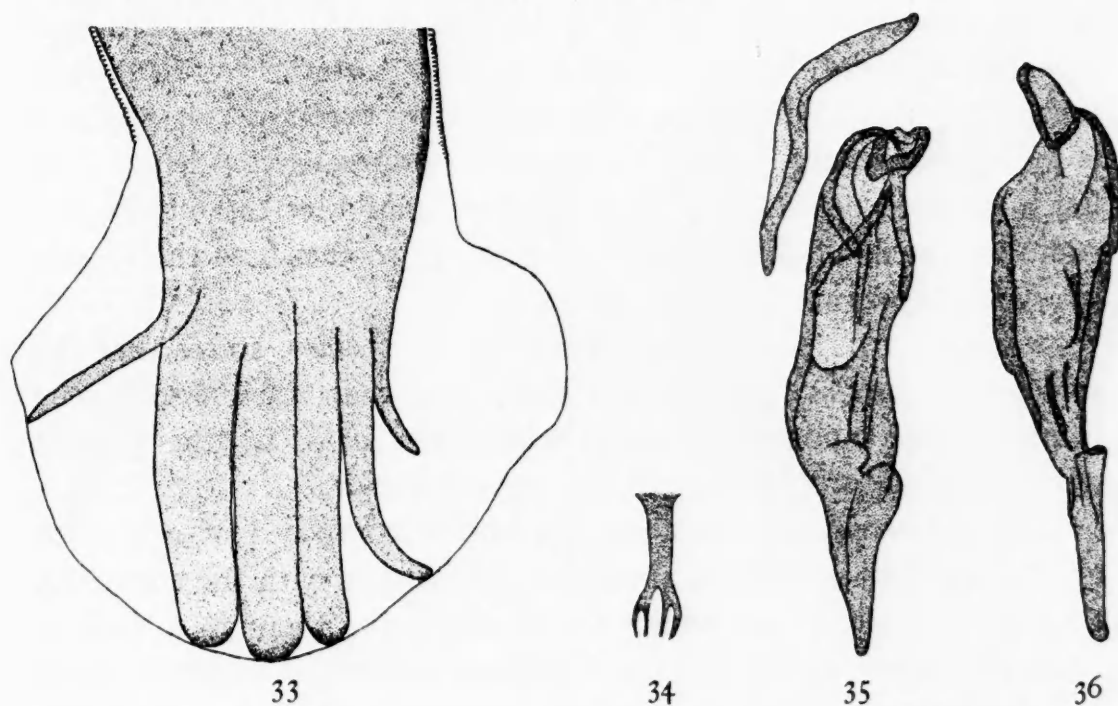
Intrauterine eggs are large, elongated and thin-shelled, and one end is broader and more rounded than the other. They measure 88 μ to 96 μ by 44 μ to 52 μ .

Host and Habitat. This species was first described from Egypt, by Looss, in 1905, from ox (*Bos taurus*); sheep (*Ovis aries* and *Ovis laticauda*); camel (*Camelus dromedarius*); goat (*Capra hircus*), and man (*Homo sapiens*). In ruminants it also occasionally occurs in the fourth stomach.

Geographical distribution. North Africa (Egypt), Europe (Great Britain), the United States of America, and British West Indies.

Trichostrongylus falculatus Ransom, 1911.

The material available for examination were two co-type males from the alimentary tract of a goat from Pesene, South Africa, kindly lent for examination by Dr. M. Hall.



H. F. Nagaty, ad nat. del.

FIGS. 33 to 36. *T. falculatus*. 33.—Bursa, left lateral view. $\times 360$. 34.—Dorsal ray, dorsal view. $\times 405$. 35.—Gubernaculum and right spicule, right lateral view. $\times 405$. 36.—Left spicule, left lateral view. $\times 405$.

The worms are small and slender. The body tapers gradually from anteriorly to the bursa. The head is provided with three inconspicuous lips and small punctiform papillae. The buccal cavity is weakly developed. There are no cervical papillae. The head measures 13μ in width. The excretory pore is 145μ to 162μ from the head end. The cuticle is transversely striated.

The male measures in the material examined 5.225 mm. to 5.662 mm. in length and 83μ to 88μ in maximum breadth anterior to the bursa. The bursal formula is as follows:—the latero-ventral and externo-lateral rays are about equal in width and wider than any of the others. Medio-lateral ray is about two-thirds the width of the externo-lateral or latero-ventral. The postero-lateral ray is narrower than the medio-lateral and its distal end is directed dorsally away from the latter. Externo-dorsal and ventro-ventral rays are narrower than any of the others, the former is comparatively short and thickened at its proximal end. The dorsal ray is bifurcate distally, each division is again divided into two; the secondary divisions are comparatively long and well developed and end in fine points.

The spicules are dark brown. The left one is slightly longer than the right, and measures 136μ in length. It has an angular process on the ventral surface at the junction of the middle and posterior thirds of the spicule, the body ends posterior to this projection in an elongated portion which gradually tapers posteriorly and is rounded at its tip. *The right spicule* measures 127μ in length and has a rounded process also at about the junction of the middle and posterior thirds of the spicule. Posteriorly to this boss the spicule gradually tapers to a rounded point.

Ransom (1911), in his description of the species, states that the spicules are similar in shape and size; that they are not so is seen from the accompanying figures, which are made by the present writer from nature, by the aid of a camera lucida.

The gubernaculum measures 70μ and is composed of a sinuous narrow band which is bent almost at right-angles with the convexity directed dorsally; posterior to this convexity there is a shallow concavity filled with a weakly chitinated material; ventrally there are two concavities having no such weakly chitinated material. Viewed dorsally the gubernaculum appears spindle-shaped.

The female worm of this species is unknown.

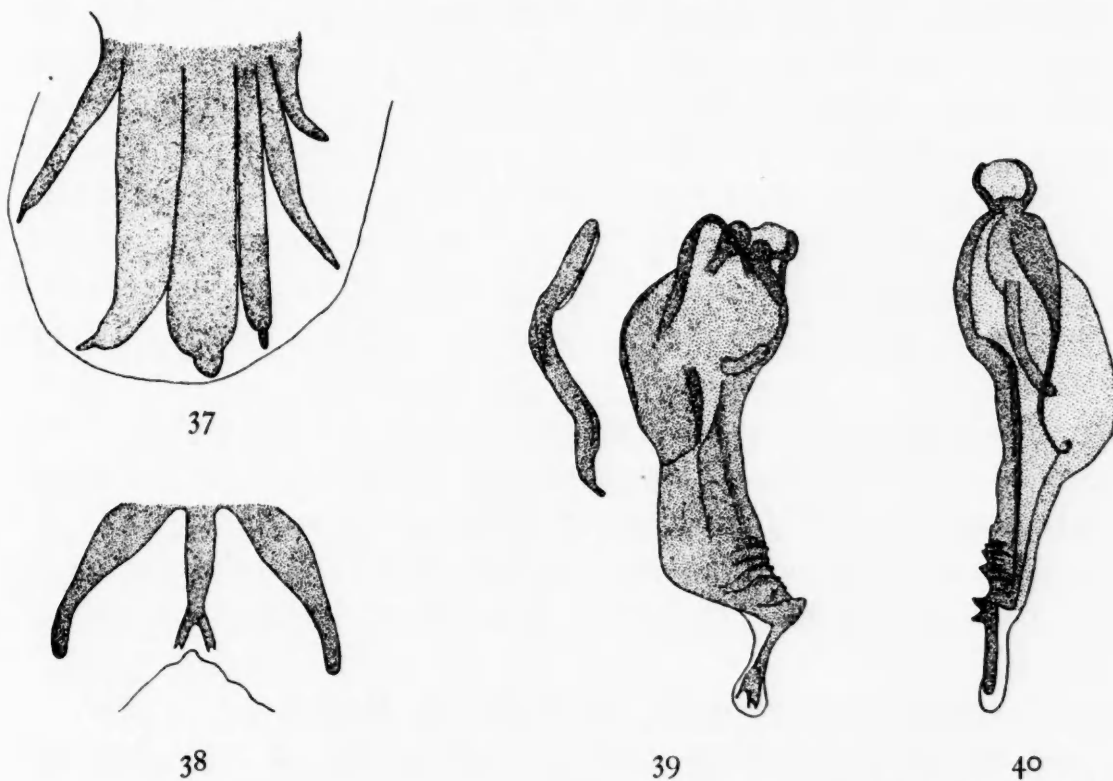
Host and Habitat. The sheep (*Ovis aries*), the goat (*Capra hircus*).

Geographical distribution. Pesene, South Africa.

Trichostrongylus rugatus Monnig, 1925.

A large amount of material from a South African sheep which suffered from a mixed infection of *T. rugatus* and *T. colubriformis* was available for examination, kindly lent for examination by Mr. A. W. N. Pillers.

The worms are small and very slender. The body is gradually attenuated anterior to the genital opening. The head is provided with three inconspicuous lips and small punctiform papillae. The buccal cavity is weakly developed. There are no cervical papillae. The head measures 13μ in width. The excretory pore is 149μ to 162μ from the cephalic end, and lies in a well-marked depression in the cuticle. The cuticle is transversely striated. The oesophagus is long and simple, measuring 761μ to 836μ in length.



H. F. Nagaty, ad nat. del.

FIGS. 37 to 40. *T. rugatus*. 37.—Bursa, left lateral view. $\times 360$. 38.—Dorsal and externo-dorsal rays, dorsal view. $\times 360$. 39.—Gubernaculum and right spicule, right lateral view. $\times 405$. 40.—Left spicule, left lateral view. $\times 405$.

The male measures 3.99 mm. to 4.845 mm. in length and 92μ to 105μ in maximum breadth in front of the bursa. The bursa has two well-developed lateral lobes but there is no distinct dorsal lobe. The bursal formula is as follows :—the ventro-ventral ray is slender and long and reaches the edge of the bursa. Latero-ventral and externo-lateral rays are of about the same width, the former ends with a narrowed extremity, while the latter ends abruptly with a small boss-like extremity, both rays reach the edge of the bursa, the former more so than the latter. The medio-lateral ray is close to the externo-lateral but is thinner and reaches the edge of the bursa. The tips of the externo-lateral and medio-lateral rays are closer together than any of the others. The postero-lateral ray is thin and gradually narrows towards the distal extremity ; it diverges from the medio-lateral and reaches the edge of the bursa. The externo-dorsal ray is nearly as long as the dorsal, which measures 44μ in length, and is divided distally into two divisions, each of which is again bifid into two very thin and short divisions.

The spicules are very dark brown in colour and are very broad anteriorly. *The left spicule* measures 136μ to 149μ in length ; *the right* is 127μ to 140μ long and is broader than the left spicule anteriorly. There is a bifid angular process at the beginning of the posterior quarter of the spicules ventrally and posterior to this the spicules end in an elongated portion which is slightly enlarged at its posterior extremity. Anterior to this angular process the ventral surface of the spicules bear a series of ridges which are very characteristic of the species ; hence the name '*rugatus*' given to it by Monnig.

The gubernaculum is less chitinised than the spicules and measures 79μ to 88μ in length. Viewed laterally it appears to be a sinuous structure and viewed dorsally or ventrally it is canoe-shaped.

The female is indistinguishable from that of *T. colubriiformis*.

Host and Habitat. In the first 8-12 feet of the small intestine of sheep.

Geographical distribution. South Africa, Malaya.

This species, together with *T. colubriiformis*, is very frequent in some parts of South Africa, where both cause heavy losses among sheep.

Trichostrongylus tenuis (Mehlis, 1846) Shipley, 1909.

The material available for examination was from the caeca of a partridge, England.

The worms are small and slender. The body tapers gradually in front of the genital opening. The head is provided with three inconspicuous lips and punctiform papillae. The buccal cavity is weakly developed. There are no cervical papillae. The head measures 13μ in width. The excretory pore is situated 154μ from the cephalic extremity. The cuticle is transversely striated and the striations are especially well marked close to the anterior end of the parasite. The oesophagus is long and simple, measuring 761μ to 809μ in length.

The male measures 5.51 mm. to 6.46 mm. in length and 61μ to 74μ in maximum breadth anteriorly to the spicules. The bursa does not show a distinct dorsal lobe. The cuticle immediately in front of the bursa ventrally is always inflated into the shape of a half moon. The bursal formula is as follows: the latero-ventral and externo-lateral rays are of about the same width, the medio-lateral is narrower than the above described rays and again the postero-lateral is narrower than the medio-lateral. The externo-dorsal ray is thickened at its proximal portion but gradually tapers distally to a narrow point. The dorsal ray is 44μ in length, is bifid at its distal third and each of these divisions is again bifid, the secondary divisions being finely pointed.

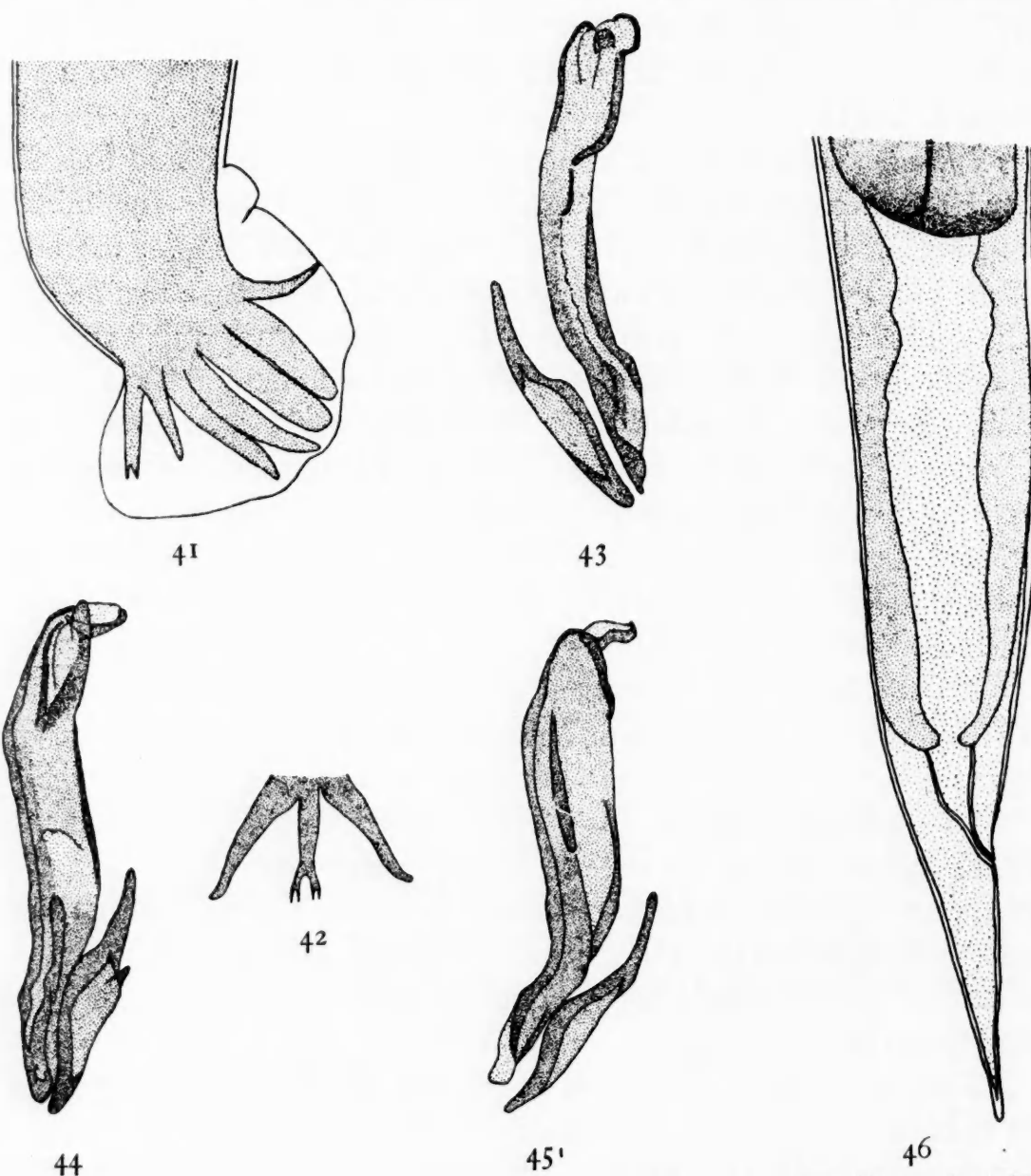
The spicules are dark brown in colour and are twisted distally. *The left spicule* is longer than the right, measuring 123.2μ to 132μ in length; *the right* measures 118μ to 123μ in length.

The gubernaculum measures 66μ in length and is less chitinised in the middle than at the edges. When viewed ventrally or dorsally it appears spindle-shaped with an anterior long and narrow portion and a posterior short and broad part. There is, on the right side only, a distinct protuberance between the anterior narrow part and the middle section.

The female measures 7.6 mm. to 7.9 mm. in length and 83μ to 92μ in maximum breadth in the region of the vulva. The latter is a longitudinal slit with a smooth homogeneous narrow band of cuticle around the opening. The slit of the vulva measures 57μ to 66μ in length and is not raised from the surface of the body. The vulva is 1.33 mm. to 1.463 mm. from the tip of the tail. The uteri are

divergent and the ovejectors are well developed; their combined length is 365μ . The uteri in this species before joining the ovejectors are dilated into a comparatively large swelling. The posterior ovary bends forward at a distance of 228μ to 264μ from the tip of the tail.

The anus is situated at a distance of 77μ to 88μ from the caudal end. The diameter of the body at the region of the anus is 22μ to 35μ . The posterior end of the body gradually narrows from the point where the posterior ovary bends forward to the tip of the tail.



H. F. Nagaty, ad nat. del.

FIGS. 41 to 46. *T. tenuis*. 41.—Bursa, right lateral view. $\times 360$. 42.—Dorsal and externo-dorsal rays, dorsal view. $\times 360$. 43.—Gubernaculum and right spicule, latero-(right) dorsal view. $\times 405$. 44.—Gubernaculum and left spicule latero-(left) dorsal view. $\times 405$. 45.—Gubernaculum and left spicule, lateral view. $\times 405$. 46.—Posterior end of female. $\times 360$.

Intrauterine eggs are thin-shelled, elongated and measure 66μ to 79μ by 35μ .

Host and Habitat. Caeca and small intestine of *Anas boschas*, *Anas boschas domesticus*, *Anser albifrons*, *Anser anser*, *Anser anser domesticus*, *Gallus gallus domesticus*, *Otis tarda*, *Perdix perdix*, *Phasianus colchicus* and *Meleagris gallopova*.

Distribution. Europe (Germany and France), Asia (Russia and Russian Turkestan), America (United States); recorded by Le Roux in the small intestine of fowl in Natal, South Africa.

Said to cause pathological effects like *T. pergracilis* in young partridges.

Trichostrongylus pergracilis (Cobbold, 1873) Shipley, 1909.

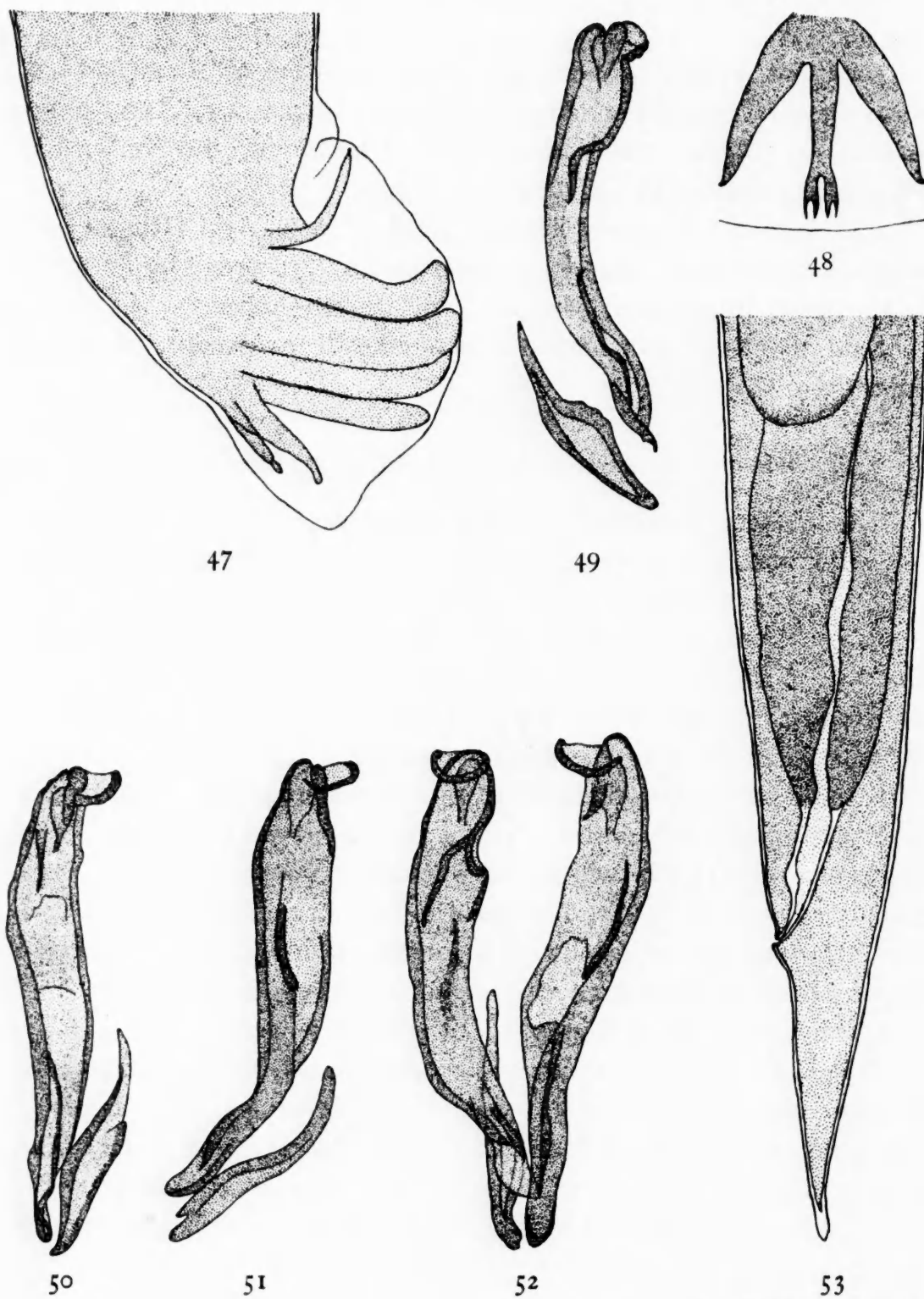
The material available for examination was :

1. From the grouse, Scotland. Six worms (three males and three females). Examined in the British Museum.
2. From the grouse. Five males and nineteen females.

Kindly lent for examination by Mr. Pillers.

The worms are small and slender. The body is gradually attenuated in front of the genital opening. The head is provided with three inconspicuous lips and punctiform papillae. The buccal cavity is weakly developed. There are no cervical papillae. The head measures 13μ in width. The excretory pore is 140μ to 171μ from the cephalic end. The cuticle is transversely striated and the striations are especially well marked a little distance behind the anterior extremity of the parasite. The oesophagus is long and simple, measuring from 712μ to 906μ in length.

The male measures 5.7 mm. to 7.41 mm. in length and 83μ to 101μ in maximum breadth in front of the spicules. The bursa is comparatively small and there is no distinct dorsal lobe. The cuticle in front of the bursa ventrally is always inflated into a half-moon shape. The bursal formula is as follows :—the latero-ventral, externo-lateral and medio-lateral are of about the same width; the externo-lateral may be slightly wider; the postero-lateral ray is narrower than the other laterals. The tips of the externo-lateral and medio-lateral are nearer together than any of the others. The externo-dorsal ray is broad at its proximal end but gradually becomes narrower towards the tip; it takes origin with the dorsal



H. F. Nagaly, ad nat. del.

FIGS. 47 to 53. *T. pergracilis*. 47.—Bursa, right lateral view. $\times 360$. 48.—Dorsal and externo-dorsal rays, dorsal view. $\times 360$. 49.—Gubernaculum and right spicule, latero-(right) dorsal view. $\times 405$. 50.—Gubernaculum and left spicule, latero-(left) dorsal view. $\times 405$. 51.—Gubernaculum and left spicule, left lateral view. $\times 405$. 52.—Spicules and gubernaculum, ventral view. $\times 405$. 53.—Posterior end of female. $\times 360$.

ray. The latter is 48μ to 66μ in length and is slightly longer than the externo-dorsal ray. The dorsal ray is bifid at its distal third and each of the divisions is again bifid; the secondary divisions end in very fine points. Shipley (1909) in his description and figures of the species must have failed to observe these secondary divisions of the dorsal ray.

The *spicules* are dark brown in colour and are much twisted, especially at their distal ends. The *left spicule* is longer than the right measuring 145μ to 162μ in length; the *right* measures 132μ to 149μ in length.

The *gubernaculum* measures 70μ to 83μ in length, and is less chitinised in the middle than at the edges. When viewed ventrally or dorsally it appears spindle-shaped with an anterior long and narrow portion and a posterior short and broad part. There is a distinct protuberance between the anterior narrow and the middle portion on the right side only.

The *female* measures from 8.949 mm. to 9.785 mm., one female only being 1.098 cm. in length and 83μ to 96μ of maximum breadth at the region of the vulva. The latter is a longitudinal slit with a smooth homogeneous narrow band of cuticle around the opening; it measures 52μ to 70μ in length and is on the same level as the general cuticle of the body. The vulva is 1.52 mm. to 1.9 mm. from the tip of the tail. The uteri are divergent and the ovejectors are well developed, their combined length is 396μ to 462μ . The posterior ovary bends forward at 264μ to 338μ from the tip of the tail. The anus is situated at a distance of 83μ to 110μ from the posterior extremity. The diameter of the body at the region of the anus is 30μ to 39μ . The posterior end of the worm gradually narrows from the point where the posterior ovary bends forward to the tip of the tail.

Intrauterine eggs are thin-shelled, elongated and measure 83μ to 88μ by 39μ to 44μ .

Host and Habitat. In the caeca of the grouse and quail.

Geographical distribution. Europe (Great Britain), United States.

This species (*T. pergracilis*) is almost identical with *T. tenuis* (Mehlis, 1846) Shipley, 1909, but differs from it in being slightly larger in size; the scarcity of good material of *T. tenuis* available for examination is the only handicap to decide whether these species

are one and the same or two distinct species. The matter of size may be explained—in case they should be identical morphologically—a fact which appears to me to be true—on the ground that the species flourishes better in the grouse than in other hosts.

This parasite is the cause of the grouse disease which causes heavy losses among the adult grouse in England and Scotland.

Trichostrongylus affinis Graybill, 1924.

Five paratype worms of this species were available for examination, two of which were males kindly lent for examination by Dr. M. Hall.

The worms are small and slender. The body tapers gradually anteriorly to the genital opening. The head is provided with three inconspicuous lips and small punctiform papillae. The buccal cavity is weakly developed. There are no cervical papillae. The head measures 13μ in width. The excretory pore is 149μ to 162μ from the cephalic extremity, and lies in a depression in the cuticle. The cuticle is transversely striated with very fine rings.

The male measures 5.225 mm. to 6.84 mm. in length and 110μ to 136μ in maximum breadth in front of the bursa.

The bursa has two lateral lobes which are united dorsally. There is no distinct dorsal lobe. The bursal formula is as follows:—the externo-lateral ray is very broad, next in breadth is the latero-ventral which is about half as broad as the externo-lateral. The medio-lateral and the postero-lateral are of about the same width and are approximately one-fourth that of the externo-lateral ray. The externo-dorsal is slightly longer than the dorsal and is thickened proximally; distally it is of the same breadth as the ventro-ventral ray. The dorsal ray ends posteriorly in two simple divisions.

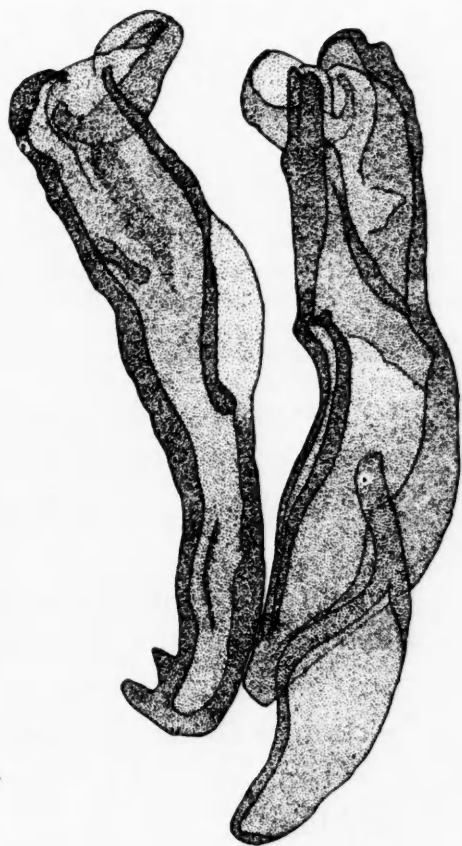
The spicules are very dark brown in colour, large and stout in comparison with those of other species of the genus except *T. calcaratus*. They are about equal in length and also bear some resemblance to each other; the left spicule is slightly longer than the right. The former measures 162μ to 184μ in length, the latter 145μ to 176μ in length; the maximum width of both spicules is 44μ . Both are slightly bent ventrally and truncate posteriorly. Viewed laterally the left spicule bears posteriorly on the ventral edge two well-developed blunt processes, the posterior of which is the larger.



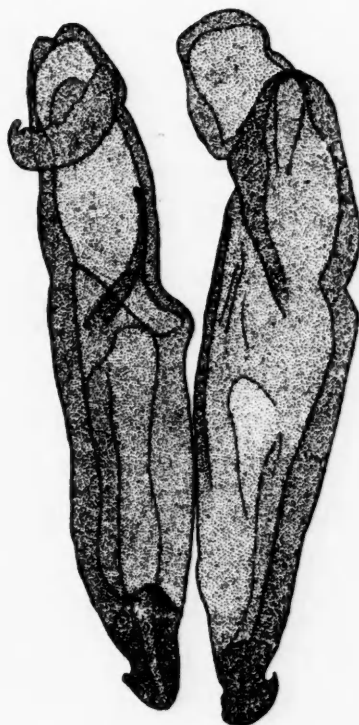
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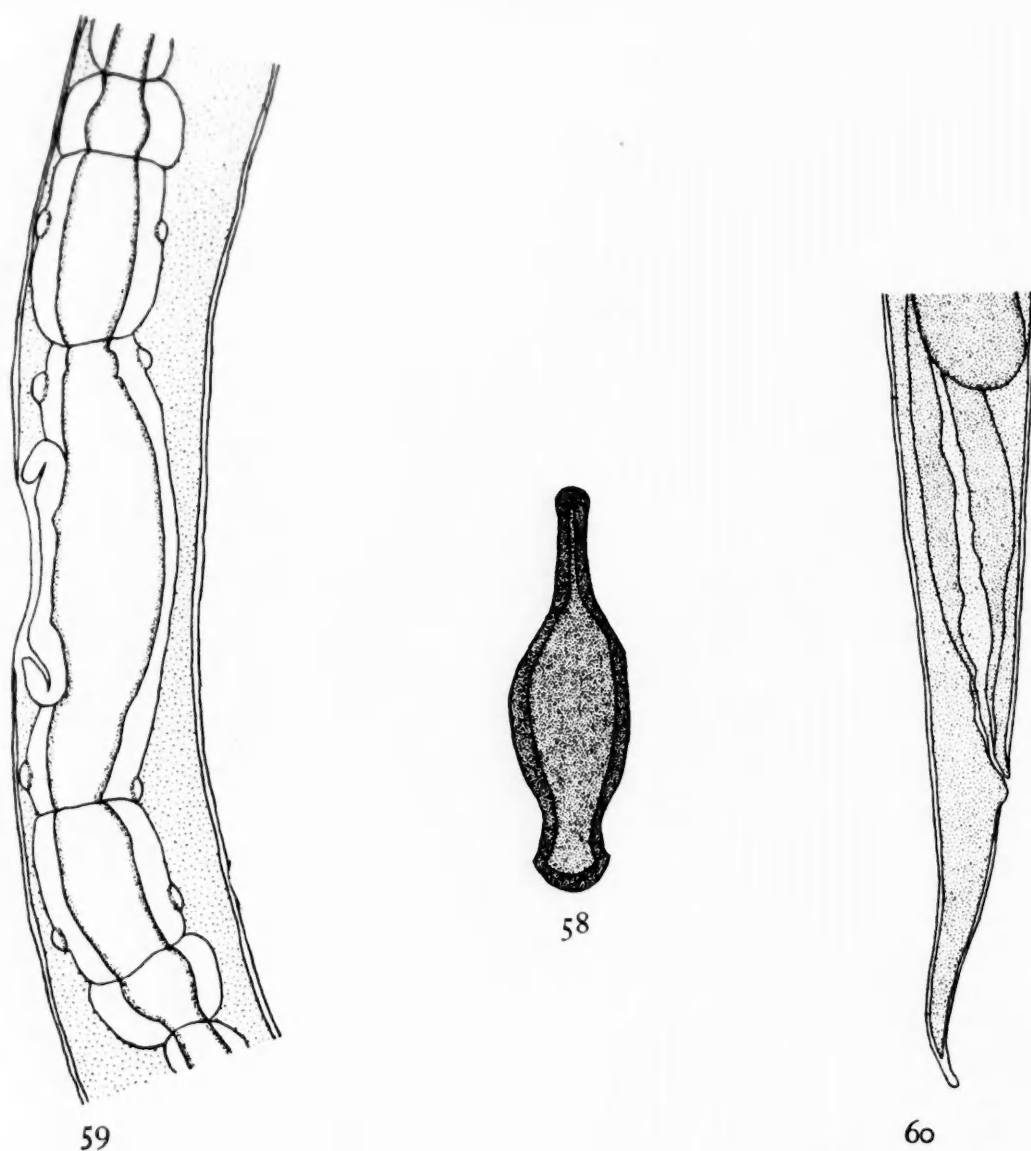
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FIGS. 54 to 60. *T. affinis*. 54.—Bursa, latero-(right) dorsal view. $\times 360$. 55.—Gubernaculum and right spicule, right lateral view. $\times 405$. 56.—Spicules and gubernaculum, latero-(left) dorsal view. $\times 405$. 57.—Spicules, ventral view. $\times 405$. 58.—Gubernaculum, dorsal view. $\times 405$. 59.—Region of the vulva and ovejectors of a female. $\times 220$. 60.—Posterior end of female. $\times 360$.

The right spicule also bears posteriorly two processes at the ventral edge which are pointed at their tips. Viewed dorsally or ventrally each spicule shows on the outer side of the posterior end a small hook-like anteriorly directed process.

The gubernaculum measures 92μ to 110μ in length and is comparatively broad and is situated dorsally between the spicules. Viewed dorsally or ventrally it comprises an elongated narrow anterior and a broad shorter posterior portion with an ellipsoidal intervening part. The anterior portion is bent ventrally. It is more chitinated at the edges than in the middle.

The female measures 6.612 mm. to 7.41 mm. in length and 88μ in maximum breadth immediately in front or behind the vulva. The latter is a longitudinal slit and measures 88μ to 132μ in length and is situated in a shallow depression in the cuticle and is 1.311 mm. to 1.52 mm. from the tip of the tail. The uteri are divergent, and the ovejectors are well developed and measure 418μ to 506μ in length and bear eight large papilla-like lateral projections. The posterior ovary bends forwards at a distance of 281μ to 347μ from the tip of the tail; the anterior ovary bends backwards for a distance of about 211μ and then for a short distance turns forward again anterior to the loop; it terminates at about 1.634 mm. from the anterior extremity. This arrangement of the ovary is different from that found in any of the species I have examined, in all of which the most anterior part of the ovary is the loop.

The anus is situated at a distance of 123μ to 149μ from the tip of the tail. The diameter of the body at the region of the anus is 30μ to 35μ . The posterior end of the body tapers gradually from the loop of the posterior ovary.

Intrauterine eggs are elongated, thin-shelled and measure 74μ by 44μ .

Host. Wild rabbit.

Geographical distribution. Princeton, New Jersey.

Trichostrongylus calcaratus Ransom, 1911.

The material available for examination consisted of co-types from the small intestine of a rabbit from Bowie, Maryland, collected by Ransom, and kindly lent by Dr. M. Hall.

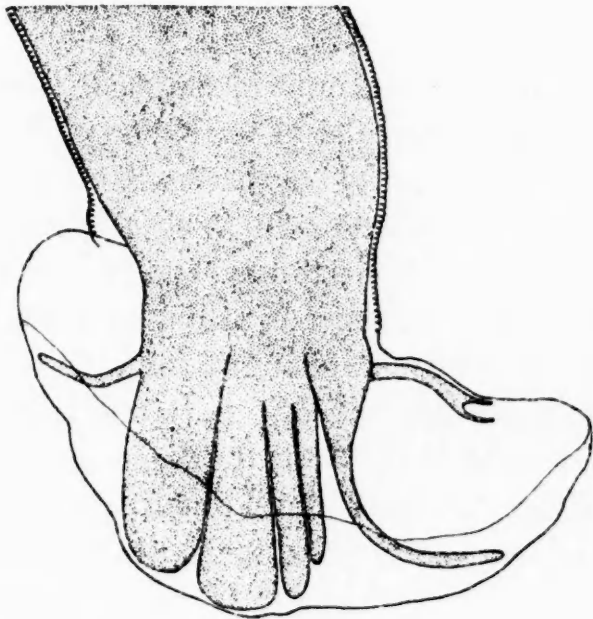
The worms are small and slender. The body is gradually attenuated anterior to the genital opening. The head is provided with three inconspicuous lips and small punctiform papillae. Buccal cavity is weakly developed. There are no cervical papillae. The head measures 13μ in width. The excretory pore is 96μ to 123μ from the cephalic end and lies in a depression in the cuticle. The cuticle is transversely striated.

The male measures, in the material examined, 3.705 mm. to 3.743 mm. in length and 83μ to 88μ in maximum breadth anterior to the bursa. The bursa has two lobes which are separated dorsally

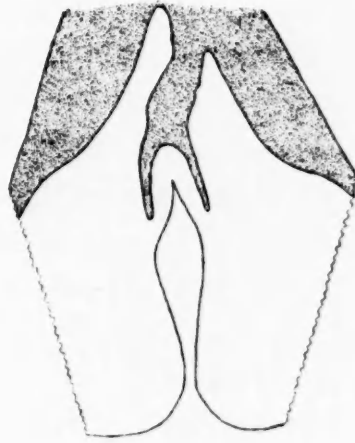
by a deep cleft, reaching as far as the two divisions of the dorsal ray. There is no dorsal lobe. The bursal formula is as follows :—latero-ventral and externo-lateral rays are the broadest, and the former is broader than the latter. The medio-lateral and postero-lateral rays are comparatively much thinner than those above described, the latter ray (postero-lateral) is thinner than the former (medio-lateral), both are less than half the thickness of the externo-lateral and about one-third that of the latero-ventral. Latero-ventral, externo-lateral, medio-lateral and postero-lateral are close together. The externo-dorsal ray is long and directed dorsally, it is much thickened in its proximal end but thin towards the distal end, which is about equal in thickness to the ventro-ventral ray. The dorsal ray is asymmetrical in that it is attached to the base of the right externo-dorsal ray. It divides distally into two slender simple divisions.

The spicules are dark brown in colour, large and stout in comparison with those of other members of the genus *Trichostrongylus*. They are almost equal in length, the left spicule is slightly longer than the right and different in shape. The former measures 176μ to 184μ in length, the latter measures 162μ to 180μ in length. Both are slightly bent ventrally and truncate at their posterior tips, unlike the spicules of other species of the genus which are pointed and angular at their posterior ends. *The left spicule* bears a spur-like process on the posterior end ventrally and a smaller process in the corresponding position dorsally. Anterior to the ventral spur-like process there is a smaller process which is directed backwards; anterior to this again there is a series of about four small bosses; these, together with the other processes above described, occupy the ventral edge of the posterior fourth of the spicule. *The right spicule* does not bear any such processes and bosses as the left except for a rod-shaped piece at the posterior end which projects slightly in pointed ends dorsally and ventrally. The tip of the posterior end in the right spicule is smaller than that of the left.

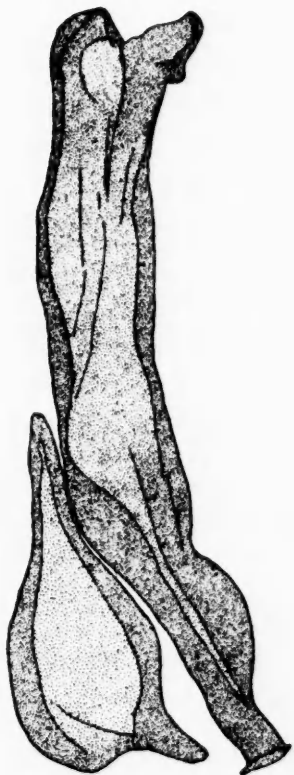
The gubernaculum measures 79μ to 88μ in length and is very broad in comparison with those in other species of the genus. It is situated dorsally in between the two spicules and is of the same colour. It is composed of a broad rather irregular middle part and an anterior and a posterior process; the former is longer and broader



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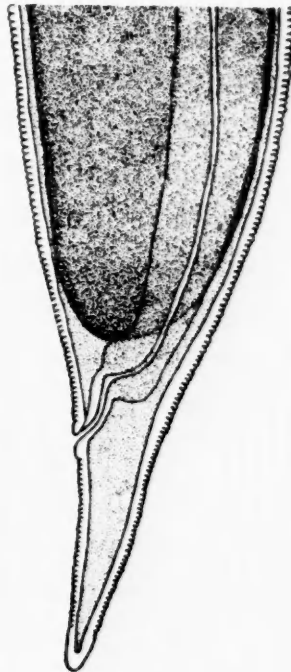
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FIGS. 61 to 65. *T. calcaratus*. 61.—Bursa, left lateral view. $\times 400$. 62.—Dorsal and externo-dorsal rays, dorsal view. $\times 400$. 63.—Gubernaculum and right spicule, right lateral view. $\times 450$. 64.—Gubernaculum and left spicule, left lateral view. $\times 450$. 65.—Posterior end of female. $\times 400$.

than the latter. The edges of the gubernaculum are more chitinised than the rest.

The female measures 4.275 mm. to 5.244 mm. in length in the material examined and 88μ in maximum breadth at the region of the vulva. The latter is covered by the anterior fold of the skin (anterior lip) and is 703μ to 722μ from the tip of the tail. The uteri are divergent and the combined length of the ovejectors is 396μ to 440μ . The anterior ovary bends backwards at about 1.463 mm. from the head end, the posterior one bends forwards at about 88μ from the tip of the tail. The anus is situated at a distance of 61μ to 66μ from the caudal extremity. The diameter of the body at the region of the anus is 22μ to 26μ . The posterior end of the body is plump and the body quickly but regularly diminishes in size posterior to the loop of the posterior ovary.

Eggs are elongated, thin shelled and measure 60μ to 70μ long by 30μ to 36μ wide.

The different measurements of *T. calcaratus* according to Ransom are as follows :—

Male. 4.7 to 6.6 mm. long, maximum thickness 100μ to 130μ at the base of the bursa. Spicules 175μ to 190μ long. Gubernaculum about 100μ by 35μ in length and breadth respectively.

Female. 5.8 to 7 mm. long by 90μ to 120μ in diameter at the region of the vulva. Anus 65μ to 90μ from the tip of the tail. Vulva 850μ to 1 mm. from the tip of the tail. Combined length of the muscular portions of the ovejectors 450μ to 560μ .

This species does not conform to the other species of the genus *Trichostrongylus* in two important points :—

1. The asymmetry of the dorsal ray and the simple tips of the bifurcations, and
2. In the shape of the distal ends of the spicules.

It is, however, doubtful if it be advisable to put this species in a new genus of its own, at least at the present time.

Host and Habitat. In the small intestine of the rabbit (*Sylvilagus floridanus mallurus* (*Lepus sylvaticus*)).

Geographical distribution. Bowie, Maryland, United States of America.

Trichostrongylus probolurus (Railliet, 1896) Looss, 1905.

Male 4.5 to 5.5 mm. long, and about 80 μ wide at the beginning of the bursa. The latero-ventral ray is by far the widest of the rays. The externo-lateral ray wider than the medio-lateral ray. The postero-lateral and externo-dorsal rays are remarkably short and close together. The end of the former is turned back so far dorsally that the postero-lateral papilla on the inner side of the bursa is directly opposite to or even somewhat dorsal of the externo-dorsal papilla upon the outer side of the bursa. Stem of the dorsal ray very short, divided as in *T. instabilis*. Spicules 126 μ to 134 μ long and relatively thick. Gubernaculum 75 μ to 80 μ long, usually of a brilliant deep-brown colour. Terminal hook of the spicule very high and sharply defined. In front of it on the ventral edge of the spicule is a second angular pointed projection, which together with the terminal hook, when observed under low magnification, gives the spicule a gnarled or twisted appearance.

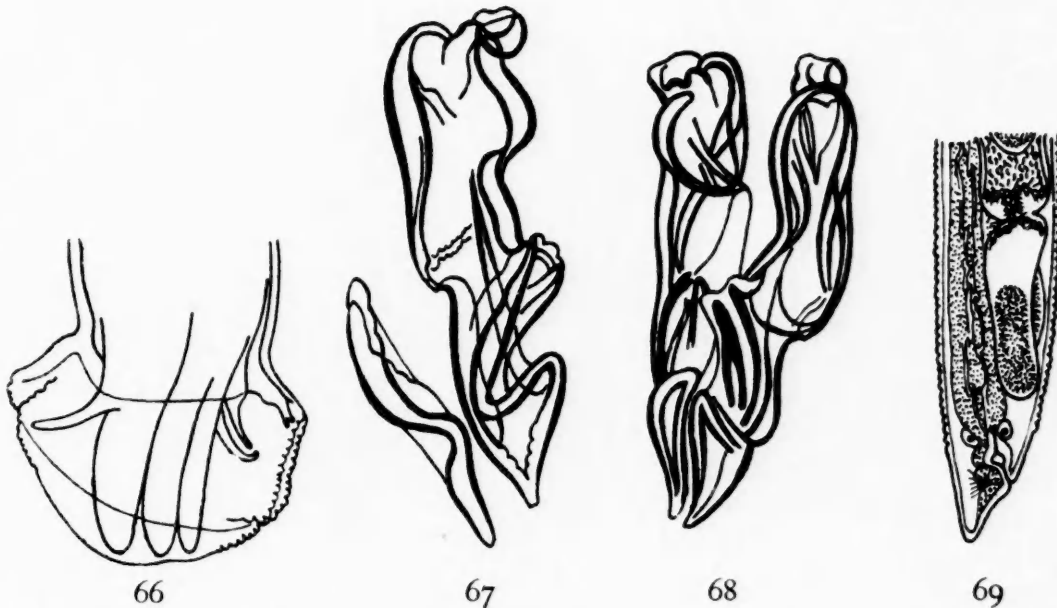
Female 4.5 to 6 mm. long and about 80 μ wide at the level of the vulva (1.08 to 1.25 mm. from the tail according to age). Posterior portion of the body rather plump, becomes narrower, beginning a very short distance in front of the anus, and rapidly diminishes in thickness, thus forming a short rather blunt tail, which commonly is turned diagonally upwards, or may be straight, or bent ventrally. Distance between tip of tail and anus 40 μ to 50 μ . Vulva including its chitinous border 76 μ long, elongated longitudinally, straight or slightly curved. Combined length of muscular portions of ovejectors including the sphincters about 375 μ . Eggs 76 μ to 80 μ long by 43 μ to 46 μ wide.

Hosts. Sheep (*Ovis aries*); Dorcas gazelle (*Gazella dorcas*); man (*Homo sapiens*); Arabian camel (*Camelus dromedarius*); Bactrian camel (*Camelus bactrianus*).

Location. Duodenum.

Localities collected. N. and E. Africa, Europe, United States.

This species has been found but once in the United States, having been collected from a camel which died at the National Zoological Park, Washington, D.C.



FIGS. 66 to 69. *T. probolurus* (after Looss). 66.—Male bursa, left lateral view. 67.—Gubernaculum and right spicule, right lateral view. 68.—Spicules, ventral view. 69.—Posterior end of female.

New locality. India.

The material for this species, seen by the writer in the British Museum (Natural History), consisted of one male.

The material was labelled *T. colubriform* (Giles, 1892). *Types*. Sheep, Punjab (Lt.-Col. Giles).

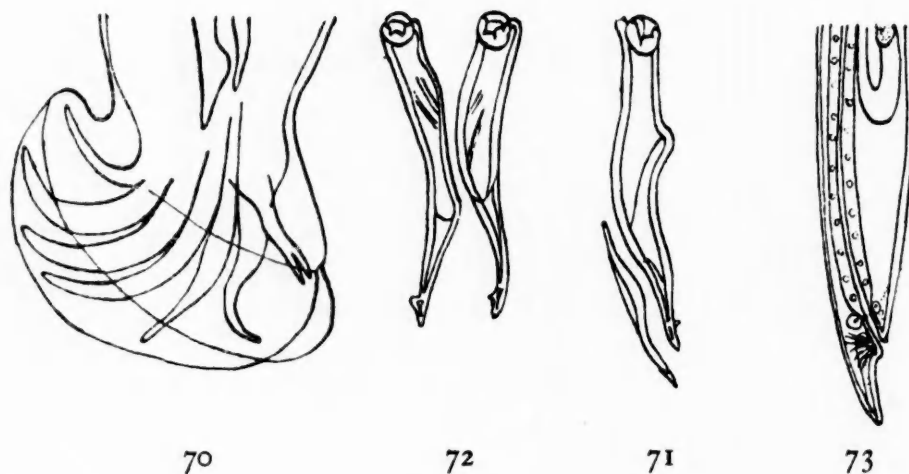
On examination, it was found to be a mixed infection of *T. colubriformis* and *T. probolurus*.

The writer believes that this is the first time *T. probolurus* has been recorded from India.

Trichostrongylus orientalis Jimbo, 1914.

SYNONYM: *Strongylus subtilis* Looss, 1895 *pro parte*.

This species of *Trichostrongylus* is not uncommon among the agricultural populations of Japan, Korea, Formosa and Central and South China. Kalantarjan (1927) has also found this infection in Armenians. It is the only species of the genus originally discovered as a human infection. The author [Faust, E. C.] has also found this species in fat-tailed sheep and Bactrian camels in North China. The trichostrongylid originally reported by Ogata, by Ijima, and by Kitamura and Oishi from human cases in Japan and Korea under the name *Strongylus subtilis* Looss, 1895, is undoubtedly referable to *T. orientalis*. Jimbo records the infection from 219 individuals and from 27 autopsies. In most cases only a few worms were present, exceptionally 50 or more. The common seat of infection was found to be the duodenum, but occasionally worms had wandered into the adjacent portion of the stomach or the jejunum. The adult worms are greyish-white in colour, the males measuring 3.8 mm. to 4.8 mm. and the females 4.9 mm. to 6.7 mm. long. The heads of the males average 7μ in diameter, and of the females, 9μ , while the greatest diameter of the former is 72μ to 79μ , and of the latter, 75μ to 83μ .



FIGS. 70 to 73. *T. orientalis* (after Faust). 70.—Bursa of male worm. $\times 150$. 71.—Gubernaculum and right spicule, right lateral view. $\times 150$. 72.—Spicules, ventral view. $\times 150$. 73.—Posterior end of female worm. $\times 150$.

The bursa is bipartite. The three lateral rays are close to one another, the latero-ventral being the broadest. All three are bowed ventrad, as is also the more slender postero-lateral. The externo-dorsal is somewhat S-shaped. The dorsal ray is bifurcated at its extremity. The two spicules measure 119μ to 133μ long, and are brownish-yellow in colour. There is a distinct minute hook at the end of each spicule. The gubernaculum measures 65μ to 85μ in length; in front view it resembles a pen nib, but in profile view it is spindle-shaped, with a slight bowing. The posterior end of the female is conical, with a graceful inward curving toward the caudal extremity. The distance from the anus to the tip of the tail is 65μ to 86μ , with a slight ventral curve. The eggs measure 75μ to 91μ in length by 39μ to 47μ in lesser diameter.

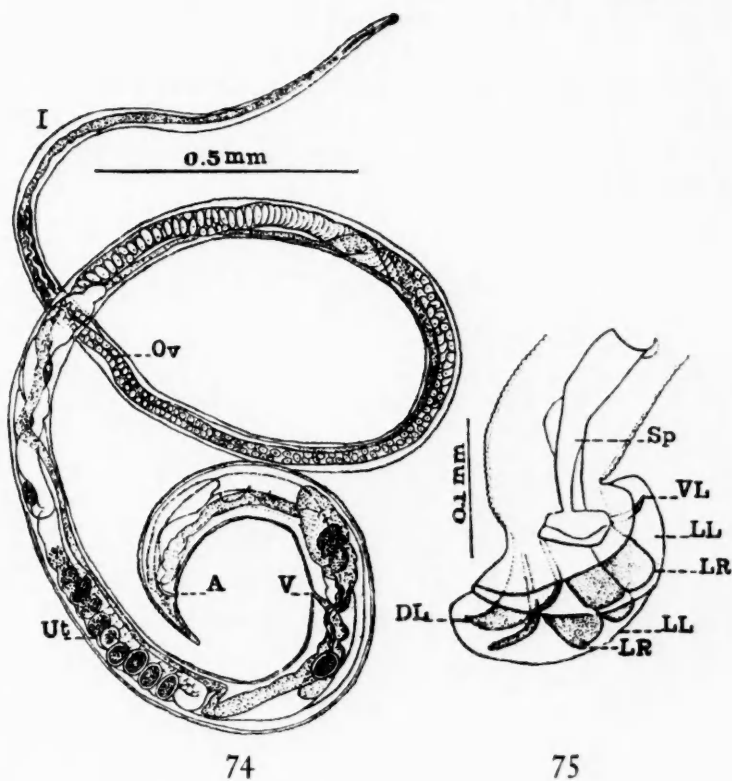
The life cycle of this worm is similar to that of *T. colubriformis*. The clinical symptoms in mild infections are nil. Carbon tetrachloride, as administered in hookworm infection, is a specific therapeutic. Man appears to be the common natural host of this species, while other mammals are only incidentally infected.

SPECIES NOT SUFFICIENTLY KNOWN WHICH MAY BELONG TO THE GENUS *TRICHOSTRONGYLUS*

Trichostrongylus fiberius Barker and Noyes, 1915.

Body thread-like, anterior region greatly attenuated, body gradually widens toward posterior end. Buccal cavity and teeth absent.

Male 2.8 mm. long; width just posterior to mouth, 0.013 mm. anterior to bursa, 0.09 mm. Bursa with two wide lateral lobes and narrow dorsal median lobe.



FIGS. 74 and 75. *T. fiberius* (after Barker and Noyes). 74.—Female worm.
75.—Posterior end of male.

Lateral lobes with two wide, blunt, lateral rays and one narrow, pointed dorso-lateral and one ventro-lateral ray. Spicules short and heavy.

Female 4.7 mm. long; width posterior to mouth, 0.03 mm., at level of vulva, 0.135 mm. Vulva in posterior ninth of body, 0.52 mm. from end. Anus 0.08 mm. from posterior end. Posterior end slightly curved and pointed. Eggs oval, segmented, 0.059 mm. by 0.036 mm., shell, thick.

Found in duodenum and caecum of the American muskrat (*Fiber zibethicus*).

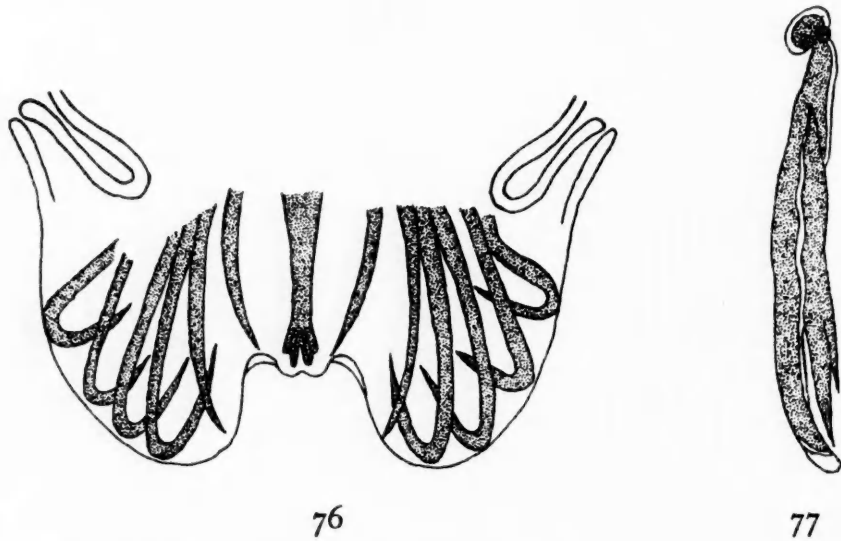
The above is the inadequate description of the species taken from the original article.

Strongylus pigmentatus von Linstow, 1904.

From stomach of hare, *Lepus nigricollis*, Cuv., Ranna, Southern Province, Ceylon.

This species is allied to *Strongylus retortæformis* Zed. The cuticle is annulate; the head trilabiate, each lip carrying on its summit a small papilla; the oesophagus occupies in the male 1/14th, in the female 1/18th of the total length; the tail is acuminate; oesophagus, testes, uterus, eggs, and especially the ovaries are coloured black.

Size of the male 7.7 mm. by 0.12 mm.; the spicula are short, 0.68 mm.; they are pale brown, and have at the root a scutiform appendix; they terminate behind in three branches, of which the largest is rounded, the others pointed; the bursa has two strong lateral lobes and a very narrow median lobe; the latter is supported by a rib which bifurcates, and each branch again divides into two branchlets which lie close beside one another; the outer branches are the shorter; the lateral lobes of the bursa are each supported by six slender ribs. The female is 10.5 mm. long by 0.14 mm.; the tail is 1/66th of the total length; the vulva is situated posteriorly, dividing the body in the proportion of 37:11; the eggs are 0.062 mm. and 0.036 mm. broad.



FIGS. 76 and 77. *S. pigmentatus* (after von Linstow). 76.—Bursa of male. 77.—Spicule

The species differs from *Strongylus retortæformis* Zed., in the formation of the head, the pigment, the cirri or spicula, and the position of the vulva. The spicula of *S. retortæformis* end in a point and are undivided and twisted; the vulva of this species is only 0.75 mm. distant from the end of the tail, in contrast with *S. pigmentata*, where it lies 2.46 mm. from the caudal extremity, the two species being approximately equal in size.

The above description and figures are copied from the original article by von Linstow.

Hall (1916) includes this species in the genus *Trichostrongylus*. With such an inadequate description the writer is not at present inclined to include this species in the genus for important reasons:—

1. That von Linstow, in his figure of the bursa, illustrates the ventro-ventral ray close to the latero-ventral ray; this is atypical of the genus.
2. In the text he states that the formation of the head is different from that in *T. retortæformis*, which is again atypical of the genus, because all the heads of the different species are as far as can be ascertained similar.

TABLE I.

Giving measurements of the different organs in *T. retortaeformis*.

	Sex	Length in mm.	Diameter of the head in μ	Distance of excretory pore from head in μ	Length of oeso- phagus in μ	Distance of vulva from tip of tail in mm.	Length of the slit of vulva in μ	Diameter of body at region of vulva in μ	Combined lengths of ovectors in μ	Distance between bend of posterior ovary and tip of tail in μ
1	♂	7.2	12	147	828
2	♂	5.96	10	?	?
3	♂	6.9	18	?	?
4	♀	7.7	18	?	?	1.5	...	90	497	...
5	♀	7.7	18	?	874	1.6	...	93	493	...
6	♀	7.9	14	115	?	1.8	...	97	540	270
7	♀	8.1	14	136	846	1.5	...	108	468	...
8	♂	6.1	14	136	792
9	♂	5.619	14	108	720
10	♂	6.372	14	151	756
11	♂	5.349	14	82	738
12	♀	6.8	14	133	?	1.278	...	79	396	...
13	♀	6.9	14	144	738	1.375	...	82	450	...
14	♀	7	14	129	817	1.5	...	86	486	...
15	♀	6.16	14	97	?	1.227	...	72	396	...
16	♀	5.353	18	115	?	1.17	...	72	396	...
17	♂	5.922	18	90	666
18	♂	6.376	14	108	774
19	♂	6.05	13	132	726
20	♂	5.49	13	149	704
21	♂	6.377	17	110	792
22	♂	6.156	17	114	800

Nos. 1-7. Material from the small intestine of mountain hare, Scotland. Examined in the British Museum.

Nos. 8-16. Material from a rabbit. Locality unknown. Kept in the Museum of the Liverpool School of Tropical Medicine.

TABLE I.

Giving measurements of the different organs in *T. retortaeformis*.

	Sex	Length of tail in μ	Diameter of body at region of anus in μ	Diameter of body anterior to spicules in μ	Length of left spicule in μ	Length of right spicule in μ	Length of gubernaculum in μ	Length of dorsal ray in μ	Length of bursa, dorso-ventrally in μ
1	♂	90	144	126	40
2	♂	82	133	115	50
3	♂	86	144	144	68
4	♀	90	39
5	♀	90	39
6	♀	108	36
7	♀	90	39
8	♂	90	144	126	72
9	♂	72	144	126	68
10	♂	100	144	126	72
11	♂	108	147	133	75
12	♀	90	28
13	♀	108	36
14	♀	126	28
15	♀	82	32
16	♀	82	32
17	♂	86	140	126	72
18	♂	93	140	126	72
19	♂	79	136	123	66
20	♂	96	145	132	70
21	♂	92	158	136	70
22	♂	92	132	118	70

Nos. 17-44. Material from a hare, from Suffolk, England. Kindly lent for examination by A. W. N. Pillers, Esq., F.R.C.V.S.

TABLE I—continued.

Giving measurements of the different organs in *T. retortaeformis*.

	Sex	Length in mm.	Diameter of the head in μ	Distance of excretory pore from head in μ	Length of oeso- phagus in μ	Distance of vulva from tip of tail in mm.	Length of the slit of vulva in μ	Diameter of body at region of vulva in μ	Combined lengths of ovectors in μ	Distance between bend of posterior ovary and tip of tail in μ
23	♂	5.559	17	127	752
24	♂	5.631	17	127	726
25	♂	6.867	17	154	800
26	♂	5.559	13	114	708
27	♂	5.396	17	132	690
28	♂	5.788	17	123	761
29	♂	6.403	13	110	756
30	♂	6.475	13	140	800
31	♂	5.833	17	114	717
32	♂	5.567	13	127	721
33	♂	5.7	13	114	?
34	♂	6.137	13	110	770
35	♀	6.39	14	144	666	1.224	...	82	396	...
36	♀	6.3	14	108	657	1.235	...	74	374	...
37	♀	7.84	17	?	880	1.496	...	96	506	...
38	♀	6.998	17	114	?	1.463	...	83	494	...
39	♀	6.156	13	110	770	1.197	...	66	418	...
40	♀	6.631	17	140	?	1.349	...	96	396	...
41	♀	7.03	17	110	?	1.52	...	83	492	...
42	♀	6.441	17	118	?	1.7	74	88	514	...
43	♀	6.403	17	110	748	1.349	66	88	462	...
44	♀	5.985	17	101	1.066 mm.	1.425	66	79	492	...

Nos. 17-44. Material from a hare, from Suffolk, England.

TABLE I—continued.

Giving measurements of the different organs in *T. retortaeformis*.

	Sex	Length of tail in μ	Diameter of body at region of anus in μ	Diameter of body anterior to spicules in μ	Length of left spicule in μ	Length of right spicule in μ	Length of gubernaculum in μ	Length of dorsal ray in μ	Length of bursa, dorso- ventrally in μ
23	♂	83	132	123	66
24	♂	79	132	123	70
25	♂	92	149	132	74
26	♂	83	132	123	66
27	♂	110	158	140	79
28	♂	88	138	127	66
29	♂	101	140	127	74
30	♂	96	145	132	70
31	♂	105	149	136	74	44	...
32	♂	83	136	118	70	39	123
33	♂	92	145	132	74	35	136
34	♂	88	149	132	70
35	♀	90	36
36	♀	92	35
37	♀	110	35
38	♀	96	35
39	♀	101	30
40	♀	118	39
41	♀	105	39
42	♀	123	37
43	♀	101	35
44	♀	88	30

Nos. 17-44. Material from a hare, from Suffolk, England.

TABLE II

Giving measurements of the different organs in *T. colubriformis*.

	Sex	Length in mm.	Diameter of the head in μ	Distance of excretory pore from head in μ	Length of oeso- phagus in μ	Distance of vulva from tip of tail in mm.	Length of the slit of vulva in μ	Diameter of body at region of vulva in μ	Combined lengths of ovectors in μ
1	♂	7.714	13	171	874
2	♂	6.004	13	158	855
3	♂	6.783	13	168	862
4	♂	5.7	13	162	751
5	♀	7.866	13	171	690	1.615	44	118	624
6	♀	8.626	13	171	774	1.786	44	110	602
7	♀	7.676	13	158	906	1.5	44	110	563
8	♀	6.346	13	154	704	1.216	44	92	497
9	♀	7.885	13	154	1.021 m.m.	1.539	44	110	598
10	♂	4.959	13	158	660
11	♂	4.883	13	132	814
12	♀	5.662	13	145	915	1.121	61	83	444
13	♀	5.092	13	114	817	1.083	44	81	396
14	♀	5.092	13	114	893	1.007	52	79	391
15	♂	4.389	13	132	?
16	♂	5.111	13	158	814
17	♂	5.206	13	140	822
18	♂	5.282	13	162	655
19	♂	5.339	13	162	790
20	♂	5.567	13	154	644
21	♂	5.833	13	158	792
22	♂	6.612	13	167	?
23	♂	6.707	13	171	805
24	♂	5.263	13	158	836
25	♂	5.605	13	154	749
26	♂	6.27	13	154	787
27	♂	6.08	13	162	902
28	♂	4.9	13	162	787
29	♂	4.9	13	158	717
30	♂	5.472	13	154	831

Nos. 1-9. Material from sable antelope (*Hippotragus niger*), Zoo, Calcutta. It is noticeable that the worms in this case are generally large (long).

Nos. 10-14. Material from the small intestine of lamb (*Ovis aries*), England; examined in the British Museum.

TABLE II

Giving measurements of the different organs in *T. colubriformis*.

	Sex	Distance between bend of posterior ovary and tip of tail in μ	Length of tail in μ	Diameter of body at region of anus in μ	Diameter of body anterior to spicules in μ	* Length of left spicule in μ	* Length of right spicule in μ	Length of gubernaculum in μ	Length of dorsal ray in μ
1	♂	92	167 (39)	154 (39)	79	...
2	♂	88	154 (39)	136 (39)	70	...
3	♂	88	167 (41)	154 (41)	79	...
4	♂	83	136 (35)	154 (39)	74	...
5	♀	176	66	44
6	♀	242	79	39
7	♀	220	74	44
8	♀	176	74	39
9	♀	184	83	48
10	♂	96	136	127	70	...
11	♂	83	132 (35)	123 (30)	61	...
12	♀	202	70	35
13	♀	255	74	30
14	♀	189	74	35
15	♂	66	154	145	74	...
16	♂	83	151 (37)	136 (37)	70	...
17	♂	83	147 (39)	134 (35)	74	...
18	♂	88	145 (37)	132 (33)	70	...
19	♂	88	149 (35)	136 (35)	70	44
20	♂	92	145 (35)	132 (39)	74	...
21	♂	79	140 (35)	132 (35)	74	39
22	♂	92	158	149	83	52
23	♂	88	167	154	83	...
24	♂	88	154 (39)	140 (39)	79	...
25	♂	95	145 (39)	132 (39)	74	...
26	♂	92	149 (37)	136 (37)	70	...
27	♂	110	154 (39)	140 (39)	70	...
28	♂	96	145 (35)	132 (35)	70	...
29	♂	92	140 (35)	123 (30)	70	...
30	♂	74	143 (35)	127 (35)	70	...

Nos. 15-45. Material from goat from Bromsgrove, England.

* The figures in parentheses indicate the position of the ventral hook from the posterior end of the spicule.

TABLE II—continued

Giving measurements of the different organs in *T. colubriformis*.

	Sex	Length in mm.	Diameter of the head in μ	Distance of excretory pore from head in μ	Length of oeso- phagus in μ	Distance of vulva from tip of tail in mm.	Length of the slit of vulva in μ	Diameter of body at region of vulva in μ	Combined lengths of ovectors in μ
31	♂	5.681	13	158	844
32	♂	6.782	13	?	?
33	♂	5.13	13	162	836
34	♂	4.693	13	145	761
35	♂	5.149	13	154	743
36	♂	5.7	13	167	809
37	♂	4.959	13	167	796
38	♂	5.282	13	154	?
39	♂	5.567	13	158	866
40	♂	4.902	13	149	743
41	♂	5.282	13	154	770
42	♂	4.883	13	154	787
43	♂	5.947	13	162	783
44	♂	5.13	13	171	870
45	♂	5.187	13	167	770
46	♂	5.415	13	162	770
47	♂	4.731	13	162	730
48	♂	5.166	13	154	787
49	♀	5.909	17	132	761	1.272	?	79	431
50	♀	6.707	13	154	756	1.397	39	92	404
51	♀	6.593	17	132	800	1.235	61	92	440
52	♀	6.745	13	140	831	1.349	44	79	466
53	♀	6.745	13	145	866	1.349	52	88	448
54	♀	6.422	13	154	765	1.406	57	96	422
55	♀	6.954	13	149	844	1.463	39	83	479
56	♀	6.688	13	132	831	1.387	52	88	470
57	♀	6.593	13	154	761	1.33	52	83	484
58	♀	6.346	13	132	893	1.33	48	83	426
59	♀	6.213	13	132	844	1.33	52	79	413

Nos. 15-59. Material from goat from Bromsgrove, England.

TABLE II—continued

Giving measurements of the different organs in *T. colubriformis*.

	Sex	Distance between bend of posterior ovary and tip of tail in μ	Length of tail in μ	Diameter of body at region of anus in μ	Diameter of body anterior to spicules in μ	* Length of left spicule in μ	* Length of right spicule in μ	Length of gubernaculum in μ	Length of dorsal ray in μ
31	♂	88	140 (35)	132 (35)	70	...
32	♂	88	158 (35)	145 (35)	72	39
33	♂	83	145 (35)	132 (30)	70	...
34	♂	79	140 (39)	123 (30)	66	...
35	♂	92	145 (35)	127 (35)	74	...
36	♂	83	149 (36)	140 (39)	70	...
37	♂	96	140 (39)	127 (35)
38	♂	88	145 (35)	132 (35)	74	...
39	♂	83	145 (35)	132 (35)	74	...
40	♂	79	140 (35)	125 (35)	66	...
41	♂	88	171 (35)	136 (35)	74	...
42	♂	88	149 (39)	132 (39)	74	...
43	♂	92	154 (39)	140 (39)	79	...
44	♂	88	149 (35)	136 (35)	74	...
45	♂	96	145 (35)	132 (35)	70	...
46	♂	88	149 (39)	136 (39)	74	...
47	♂	74	140 (35)	132 (35)	66	...
48	♂	74	149	138	74	...
49	♀	202	74	26
50	♀	180	70	35
51	♀	387 ?	83	35
52	♀	211	70	39
53	♀	233	79	35
54	♀	228	88	35
55	♀	224	79	39
56	♀	237	92	48
57	♀	198	83	30
58	♀	189	74	35
59	♀	211	77	30

Nos. 15-59. Material from goat from Bromsgrove, England.

* The figures in parentheses indicate the position of the ventral hook from the posterior end of the spicule.

TABLE III.

Giving measurements of the different organs in *T. capricola*.

	Sex	Length in mm.	Diameter of the head in μ	Distance of excretory pore from head in μ	Length of oeso- phagus in μ	Distance of vulva from tip of tail in mm.	Length of the slit of the vulva in μ	Diameter of body at region of vulva in μ	Combined lengths of ovejectors in μ
1	♂	3.552	13	132	660
2	♂	3.656	13	110	?
3	♀	5.605	13	140	682	1.045	61	83	352
4	♀	5.557	13	110	598	1.045	44	74	334
5	♂	4.541	13	167	796
6	♂	5.149	13	162	875
7	♂	4.617	13	145	972
8	♂	5.89	13	180	897
9	♂	4.845	13	154	849
10	♂	4.541	13	167	800
11	♂	4.883	13	167	717
12	♂	5.377	13	132	?
13	♂	4.997	13	123	?
14	♂	5.738	13	167	946
15	♂	4.436	13	162	844
16	♂	4.655	13	162	814
17	♂	3.629	13	136	897
18	♂	6.023	13	158	853
19	♂	5.396	13	167	871
20	♂	5.13	13	140	932
21	♂	4.769	13	118	893
22	♂	5.244	13	167	857
23	♂	3.724	13	149	756
24	♂	5.130	13	158	818
25	♂	6.042	13	158	902
26	♂	5.689	13	154	822
27	♂	5.662	13	167	726
28	♂	5.339	13	158	928
29	♂	5.244	13	162	1.008 mm.
30	♂	4.199	13	149	884
31	♂	4.598	13	145	831
32	♀	6.479	17	145	836	1.178	44	92	378
33	♀	5.054	13	132	919	1.074	52	92	360
34	♀	6.08	13	154	902	1.235	52	83	365
35	♀	6.061	13	132	618	1.159	48	83	391

Nos. 1-4. Material from *Capra bircus* (Maltese), 4th stomach.

TABLE III.

Giving measurements of the different organs in *T. capricola*.

	Sex	Distance between bend of posterior ovary and tip of tail in μ	Length of tail in μ	Diameter of body at region of anus in μ	Diameter of body anterior to spicules in μ	Length of left spicule in μ	Length of right spicule in μ	Length of gubernaculum in μ	Length of dorsal ray in μ
1	♂	66	132	132	70	...
2	♂	66	136	136	74	...
3	♀	?	52	35
4	♀	145	74	30
5	♂	74	121	121	66	...
6	♂	96	145	134	74	...
7	♂	74	132	127	74	...
8	♂	92	149	145	83	44
9	♂	92	140	136	74	...
10	♂	96	140	136	74	39
11	♂	83	132	132	74	...
12	♂	79	136	136	81	48
13	♂	92	145	145	88	48
14	♂	110	140	140	79	...
15	♂	88	136	136	79	...
16	♂	96	140	127	74	...
17	♂	74	123	118	70	...
18	♂	105	140	136	74	...
19	♂	88	132	118	74	...
20	♂	96	140	140	79	...
21	♂	92	136	136	74	36
22	♂	88	136	136	74	...
23	♂	74	118	114	83	...
24	♂	88	132	127	79	...
25	♂	74	149	149	88	48
26	♂	96	145	132	79	...
27	♂	96	145	123	79	...
28	♂	101	140	132	79	...
29	♂	83	140	132	74	...
30	♂	79	136	132	74	...
31	♂	79	127	118	74	...
32	♀	193	74	35
33	♀	167	72	39
34	♀	250	88	30
35	♀	198	96	35

Nos. 5 to 35. Material from the small intestine of a goat from Bromsgrove, England.

TABLE IV.

Giving measurements of the different organs in *T. vitrinus*, *T. rugatus*, *T. tenuis*, *T. pergracilis*, *T. affinis* and *T. calcaratus*.

	Sex	Length in mm.	Distance of excretory pore from head in μ	Length of oeso- phagus in μ	Distance of vulva from tip of tail in mm.	Length of the slit of vulva in μ	Diameter of body at region of vulva in μ	Combined lengths of ovejectors in μ	Distance between bend of posterior ovary and tip of tail in μ
1	♂	6.023	?	752
2	♂	7.296	?	874
3	♂	6.27	176	?
4	♂	5.605	167	708
5	♂	7.125	180	?
6	♀	8.132	176	?	1.577	?	88	440	?
7	♀	7.315	?	?	1.5	57	79	413	321
8	♀	7.695	145	770	1.539	74	96	409	264
9	♀	6.935	154	770	1.444	44	92	404	294
10	♀	6.897	154	748	1.406	70	79	484	277
11	♂	4.446	154	761
12	♂	4.845	154	836
13	♂	4.085	149	?
14	♂	4.503	162	822
15	♂	3.99	154	?
16	♂	5.51	154	?
17	♂	6.175	154	?
18	♂	6.46	?	809
19	♀	7.923	?	792	1.33	57	92	365	228
20	♀	7.6	?	761	1.463	66	83	365	264

Nos. 1-10. Material from the small intestine and stomach of sheep, England.

Nos. 11-15. Material from a sheep, South Africa.

TABLE IV.

Giving measurements of the different organs in *T. vitrinus*, *T. rugatus*, *T. tenuis*, *T. pergracilis*, *T. affinis* and *T. calcaratus*.

	Sex	Length of tail in μ	Diameter of body at region of anus in μ	Diameter of body anterior to spicules in μ	Length of left spicule in μ	Length of right spicule in μ	Length of gubernaculum in μ	Length of dorsal ray in μ	
1	♂	101	149	149	79	66	} <i>T. vitrinus</i>
2	♂	92	176	162	92	61	
3	♂	88	167	167	88	61	
4	♂	79	149	149	74	52	
5	♂	92	176	171	96	...	
6	♀	83	39	
7	♀	90	39	
8	♀	92	39	
9	♀	79	35	
10	♀	83	35	
11	♂	101	136	127	79	...	} <i>T. rugatus</i>
12	♂	96	149	140	83	...	
13	♂	105	140	132	83	...	
14	♂	101	149	140	88	44	
15	♂	92	140	132	79	...	
16	♂	74	123	118	66	44	} <i>T. tenuis</i>
17	♂	66	132	123	66	44	
18	♂	61	132	118	66	44	
19	♀	88	35	
20	♀	77	22	

Nos. 16-20. Material from the caeca of partridge, England.

TABLE IV—continued.

Giving measurements of the different organs in *T. vitrinus*, *T. rugatus*, *T. tenuis*, *T. pergracilis*,
T. affinis and *T. calcaratus*.

	Sex	Length in mm.	Distance of excretory pore from head in μ	Length of oeso- phagus in μ	Distance of vulva from tip of tail in mm.	Length of the slit of vulva in μ	Diameter of body at region of vulva in μ	Combined lengths of ovectors in μ	Distance between bend of posterior ovary and tip of tail in μ
21	♂	7.315	?	770
22	♂	7.049	?	756
23	♂	7.087	?	770
24	♂	6.992	?	814
25	♂	6.27	?	730
26	♀	9.785	171	778	1.52	70	88	418	264
27	♀	10.98	?	805	1.9	70	83	462	290
28	♀	9.291	?	770	1.729	66	88	404	294
29	♂	6.27	140	792
30	♂	7.41	?	787
31	♂	5.7	162	712
32	♀	8.949	?	906	1.577	66	88	396	272
33	♀	9.025	?	787	1.7	70	88	400	338
34	♀	9.5	?	880	1.52	52	96	435	308
35	♂	6.84	162	888
36	♂	5.225	154	858
37	♀	7.315	162	871	1.52	132	88	453	330
38	♀	7.41	149	818	1.444	88	88	506	347
39	♀	6.612	158	805	1.311	118	88	418	281
40	♂	3.705	96	?
41	♂	3.743	123	?
42	♀	4.275	114	?	703	...	88	396	88
43	♀	5.244	123	?	722	...	88	440	88

Nos. 21-34. Material from the grouse, Scotland.

Nos. 35-39. Material from a wild rabbit, Princeton, New Jersey.

TABLE IV—continued.

Giving measurements of the different organs in *T. vitrinus*, *T. rugatus*, *T. tenuis*, *T. pergracilis*, *T. affinis* and *T. calcaratus*.

	Sex	Length of tail in μ	Diameter of body at region of anus in μ	Diameter of body anterior to spicules in μ	Length of left spicule in μ	Length of right spicule in μ	Length of gubernaculum in μ	Length of dorsal ray in μ	
21	♂	83	154	145	70	66	} <i>T. pergracilis</i>
22	♂	79	145	132	79	57	
23	♂	83	145	132	74	61	
24	♂	96	145	132	79	57	
25	♂	76	145	132	74	48	
26	♀	88	35	
27	♀	83	30	
28	♀	105	35	
29	♂	101	154	140	83	48	
30	♂	88	149	132	79	...	
31	♂	88	162	149	83	52	
32	♀	105	30	
33	♀	110	39	
34	♀	83	30	
35	♂	136	184	176	110	...	} <i>T. affinis</i>
36	♂	110	162	145	92	...	
37	♀	145	35	
38	♀	149	35	
39	♀	123	30	
40	♂	83	184	180	88	...	} <i>T. calcaratus</i>
41	♂	88	176	162	79	...	
42	♀	61	22	
43	♀	66	26	

Nos. 40-43. Material from the small intestine of a rabbit from Bowie, Maryland, U.S.A.

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SOME FURTHER OBSERVATIONS ON *CHARA FRAGILIS* IN RELATION TO MOSQUITO BREEDING IN QUEENSLAND

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Although the general consensus of opinion seems to be that charophytes are useless for purposes of biological control of mosquitoes, Matheson and Hinman (1928-29, 1930-31) continue, with certain species of the characeae, to obtain positive results in their experiments, and to affirm and reaffirm that these algae prevent mosquitoes from breeding.

Results obtained by us, on the contrary, have been consistently of a negative nature and provide sufficient justification for a further contribution on this vexed question, especially as our most recent work has been done with *Chara fragilis*, a species hitherto said to be responsible for inhibiting larval growth in waters in which it occurs. Our experiments to which reference is made in this paper show that charophytes in most phases of growth do not prevent the breeding of the two house mosquitoes (*Stegomyia fasciata* Fabr., and *Culex fatigans* Wied.), nor the tree cavity-breeder, *Aedes notoscriptus* Skuse, from reaching maturity. There are many instances which come under one's notice, when *Chara*-containing waters do not breed mosquitoes, the reasons for which may be legion; hence here are appended positive experiments only. These were conducted mainly with domestic varieties; an opportunity to experiment with sylvan mosquitoes did not present itself.

During the early part of last year, quantities of *Chara fragilis* were obtained from large cement horse troughs at the Gatton Agricultural College, and the species was identified by Mr. James Groves, to whom the author's thanks are due. The plant, which was

in excellent fruiting condition and typical in every phase of its growth, was introduced into the laboratory during the middle of our Queensland winter. *Anopheles annulipes* had, during the summer months, been found breeding in these horse troughs situated in the middle of a large horse paddock, a typical breeding place for this species (Hamlyn-Harris, 1928).

Experiments were so arranged that adults of *Stegomyia fasciata* and *Culex fatigans* might have access to these jars containing various types of aquatic vegetation, *just how and when they pleased*. This was of immense advantage. Dozens of jars were made available to adults of these species, and it is, therefore, all the more significant when it is realized that in these experiments the only jars selected were those containing *Chara fragilis*. The control jars contained *Hydrilla*, *Ceratophyllum*, *Valisneria* and other aquatic vegetation respectively, and were kept at the same temperature and under similar conditions throughout; even the pH varied but little in any one tank, but on the whole remained consistently the same right through the experiments, which were maintained for months.

In view of these observations, it would seem futile to go on reiterating results such as these; but as these observations carry no weight unless given in detail, experiments spread over June and July of last year are here tabulated with temperature and pH records during the time that the larvae were most plentiful. It will be noted that during the whole period under review, very slight variations in temperature and pH values occurred, either in the experimental or control jars, from which it may be inferred that the vegetation, which was of a varied nature, did not appear to exercise much influence in this particular direction.

Control jars containing algal growths of various kinds, *Hydrilla*, *Ceratophyllum*, *Utricularia* and *Valisneria* respectively, were assembled close to the experimental jars. None of these jars was selected by either *Culex fatigans* or *Stegomyia fasciata* at any time. Experiments of this nature have been continued for some years; and it is strange that the only jars selected (principally by *Stegomyia fasciata*) have been jars containing either *Chara fragilis*, *Nitella phauloteles*, *Nitella gelatinosa* or *Nitella diffusa* (Hamlyn-Harris, 1930) in varying stages of development. There is not a fraction of doubt about this.

Date	EXPERIMENT NO. 1				EXPERIMENT NO. 2			
	Conducted with <i>Chara fragilis</i> in full fruiting condition.				Conducted with growing <i>Chara fragilis</i> .			
	Temperature	pH	Type of mosquito selecting	Stage of development	Temperature	pH	Type of mosquito selecting	Stage of development
28.5.31	64° F.	7.6	<i>Culex fatigans</i> ...	Eggs.	64° F.	7.6	<i>Stegomyia fasciata</i>	Larvae.
12.5.31	68° F.	7.6	<i>Culex fatigans</i> ...	Larvae.	68° F.	8.0	<i>Stegomyia fasciata</i>	Larvae and pupae.
18.6.31	61° F.	7.4	<i>Culex fatigans</i> ...	Larvae and pupae.	59° F.	7.3	<i>Stegomyia fasciata</i>	Larvae.
24.6.31	61° F.	7.4	<i>Culex fatigans</i> ...	Eggs (2 batches). Larvae and pupae.	61° F.	7.6	<i>Stegomyia fasciata</i>	Larvae.
2.7.31	63° F.	7.6	<i>Culex fatigans</i> ...	Larvae.	62° F.	7.6	<i>Stegomyia fasciata</i>	Larvae.
8.7.31	56° F.	7.8	<i>Culex fatigans</i> ...	Larvae and pupae.	57° F.	7.6	<i>Stegomyia fasciata</i>	Larvae and pupae.
15.7.31	56° F.	7.8	<i>Culex fatigans</i>	Larvae and pupae.	55° F.	7.6	<i>Stegomyia fasciata</i>	Larvae and pupae.
29.7.31	64° F.	7.8	<i>Culex fatigans</i> ...	Larvae and pupae.	64° F.	7.8	<i>Culex fatigans</i> ...	Eggs.
	Adults hatched out at intervals.				Adults hatched out at intervals.			

Date	EXPERIMENT NO. 3			
	Conducted with decomposing <i>Chara fragilis</i> .			
	Temperature	pH	Type of mosquito selecting	Remarks
28.5.31	63° F.	7.6	None	No mosquitoes selected this water until a month or so after the experiments had been abandoned, when <i>Stegomyia fasciata</i> bred freely.
12.5.31	60° F.	7.4	None	
18.6.31	61° F.	7.6	None	
24.6.31	61° F.	7.6	None	Decay arrested and fresh growth visible.
2.7.31	62° F.	7.8	<i>Aedes notoscriptus</i>	Larvae of the second instar introduced.
8.7.31	56° F.	7.8	<i>Aedes notoscriptus</i>	Larvae and pupae.
15.7.31	56° F.	7.5	<i>Aedes notoscriptus</i>	Larvae and pupae.
29.7.31	64° F.	7.8	<i>Aedes notoscriptus</i>	Larvae and pupae, all of which matured in course of time.

EXPERIMENT NO. 4 was conducted in a small glass tank containing waters which had not been changed for over six weeks; *Hydra* sp. occurred in considerable numbers. By the addition of fruiting *Chara fragilis*, a perfectly balanced water resulted, which was selected by *Culex fatigans* within a week. Within six days, larvae had reached the second, a few the third, instar. At this stage it was noticed that a good many young larvae were covered with a fungoid growth and were dying. Subsequent investigations proved the disease to be due to *Saprolegnia monica* Pringsheim, and fatal whenever the larvae became infested. About this time also, a *Hydra*, common here in Brisbane waters, became very active and took an enormous toll of larval life—I never saw a larva escape once it had been attacked and caught. Simultaneously with these two larval destructors, I noticed some larvivorous Coleopterous larvae busy simultaneously destroying mosquito larvae; these remained active long after *Saprolegnia* and *Hydra* had ceased to operate. As soon as the activity of these larval destructors had died down, several pupae commenced to hatch out, by which it may be inferred that the death of the larvae was not due to the presence of *Chara fragilis*, since surviving larvae all pupated, and egg rafts again appeared on the water two or three days afterwards. With the stimulus of a small quantity of subsidiary artificial food added to the water from time to time, the remaining larvae continued to thrive, though the period of development was retarded somewhat owing to winter conditions at the most unfavourable time of the year; their growth nevertheless appeared quite normal, males and females were produced from time to time, scattered over a period of three months.

EXPERIMENT NO. 5. Among the equipment of the laboratory, a large earthenware jar, capable of holding about two gallons of water, was used for growing various charophytes. This jar has been uninterruptedly in use for about four years; the water has never been changed, but fresh water necessarily has been added from time to time. Twelve months ago a healthy growth of *Chara fragilis* was embedded in sand, and thrived for about four months before the plant commenced to decompose; during this time the water remained undisturbed, and adults of *Stegomyia fasciata* as well as *Culex fatigans* had access to this water. Both somewhat spasmodically selected it from time to time, but *Stegomyia fasciata* produced many

more normal adults, after somewhat drawn-out developmental stages, than *Culex fatigans*.

During the whole of this time *Chara fragilis* imparted to the water a most unpleasant foetid smell, suggestive of garlic; this factor did not seem to have acted as a deterrent, for *Stegomyia fasciata* bred specially freely in a perfectly clear water containing no other plant growth of any sort. Since then the *Chara* has completely decomposed and the debris has remained at the bottom of the container; and *Stegomyia fasciata* has again selected it and bred out adults.

Observations were also made with reference to the possibility of oxygen bubbles being detrimental to the larvae (Matheson and Hinman, 1931). There seemed to be no direct evidence of this, though in one experiment I had noticed what appeared to be the detrimental effects of bubbling oxygen through water, when the mosquito larvae were to all appearances adversely affected.

In view of these facts, it may be useful to give the analysis of this water, made at the time, which I here append, in the hope that some time or other the study of these rather unusual combinations may lead to a better understanding of the problem confronting us, especially as they show clearly in what type of water *Stegomyia fasciata* will breed normally, in spite of the presence of charophytes reputed to be deleterious to the normal development of mosquito life.

ANALYSIS OF CHARA WATER.

pH	8.4-8.5.
Total alkalinity as CaCO_3	222 p.p.m.
Total hardness as CaCO_3	303 p.p.m.
Chlorides as Cl.	156 p.p.m.
Calcium as CaO	82.8 p.p.m.
Magnesium as Mgo	62.0
Oxygen consumed (30 mins. at 212°F.)	3.8
Free and Saline Ammonia (as N.)	0.020
Albuminoid Ammonia	0.140
Nitrite (as N.)	0.00075
Nitrate (as N.)...	0.036

This is intended only as a record for future use; it is not at present possible to interpret these findings in terms of the analysis.

Only in one or two instances was artificial food supplied. In most jars the larvae had to rely entirely on such food as the jar

contained. Only in the experimental jar used in Experiment No. 4 was an artificial fish food (piscidin) employed to hasten development.

These results, selected from a host of instances, speak for themselves and are in keeping with all previous and subsequent observations made by the author: they conclusively prove his contentions that *charophytes are of no value* in the biological control of mosquitoes in Queensland. (See also Twinn, 1931.) In any case it is extremely difficult to determine the reason for any particular action of the waters in which charophytes thrive or die, for guesswork must play a large part in the determination of so many obscure factors, any one of which may be responsible for the results, and about which we unfortunately at present know nothing. It cannot be denied that there are numerous instances of waters containing charophytes in which no breeding has been found to take place. The one is as much a scientific fact as the other, but to all intents and purposes such potential breeding places betray no recognisable differences from the actual ones herein referred to.

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THE INFLUENCE OF CYCLICAL TRANSMISSION BY *GLOSSINA TACHINOIDES* ON A STRAIN OF *TRYPANOSOMA BRUCEI*, MADE RESISTANT TO HUMAN SERUM

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In their paper on the action *in vitro* of normal human serum on the pathogenic trypanosomes, Yorke, Adams and Murgatroyd (1930) give an admirable resumé of earlier work. It would not perhaps be out of place to recapitulate the main points which have a bearing on the present subject.

Laveran (1902) discovered that the injection of human serum has a marked effect on the course of nagana in mice. Laveran and Mesnil (1904) showed that, although the injection of human serum causes a complete disappearance of trypanosomes from the blood of mice, this disappearance is only of a temporary nature. After some time the trypanosomes reappear, but another dose of serum will cause them to disappear again. It was pointed out that after repeated injections the serum seems gradually to lose its potency. The diminution of trypanocidal power is so slow a process that it is possible to keep an infected mouse alive for two or three months by giving appropriate doses of serum.

Jacoby (1909) used this method to make a strain of nagana in mice completely resistant to 2 c.c. of human serum. Leboeuf (1911), attempting to confirm this work, found that, although with several mice he was able to make the strain of nagana resistant to doses of human serum, passage of the resistant strain to other mice invariably caused loss of the resistance. Mesnil and Ringenbach (1911) found that *T. rhodesiense* in mice is very susceptible to the action of human serum. Laveran and Nattan Larrier (1912) showed that this

trypanosome could rapidly acquire a resistance to human serum, and that this resistance could survive numerous mouse passages.

In the paper already mentioned, Yorke and his co-workers elaborate a hypothesis regarding the epidemiology of rhodesiense and gambiense sleeping sickness. They suggest that, although *T. rhodesiense* is identical with *T. brucei*, normal human beings cannot be infected with the game trypanosome because of the great trypanocidal power of their serum. In certain pathological conditions the trypanocidal power of the blood is lost, so that individuals suffering from such conditions are susceptible to infection. As soon as the game trypanosome gets established in this way, it tends to become by degrees serum-resistant, until after prolonged sojourn in man it becomes serum-fast, and so infective to normal human beings. If this trypanosome (*T. rhodesiense*) is passed into game or domestic stock, it rapidly loses its serum-fast properties and is again incapable of infecting normal man (*T. brucei*). If, on the other hand, it is repeatedly passed by tsetse from man to man, the serum-resistance is intensified until it becomes a fixed character. In such a manner *T. rhodesiense* becomes *T. gambiense*.

If this hypothesis is correct, the serum fast character must be retained through cyclical transmission by tsetse. In this connection Yorke writes: 'The serum-fastness is possibly preserved during the passage of the parasite through *Glossina*; and if so, the infection could spread from man to man through cyclically infected tsetse. If, however, this proves not to be the case, then the only obvious explanation of such localised epidemics of this form of the disease as have occurred, e.g., the Mwanza epidemic, is either that the fly transmits mechanically the serum-fast parasite from man to man, or that some local condition exists, e.g., dietary deficiency, or hook-worm disease, which so affects the population as to deprive many individuals of the protection due to the trypanocidal power of the blood.' In the event of the serum-fast character not being preserved during passage through tsetse, it is difficult to see how *T. rhodesiense* could develop into *T. gambiense* on the lines of this hypothesis. The two alternative explanations indicate how epidemics of *T. rhodesiense* might originate from *T. brucei*, but they do not carry us any further than that.

Various workers have attempted to discover whether acquired

characters of a parasite can be preserved during cyclical passage through the invertebrate host, but their results have not been in agreement. Gonder (1911), working with a drug-resistant strain of *T. lewisi*, found that after twenty to thirty days in the louse, the parasite had lost its drug-resistance. Duke (1927) criticises these findings on the grounds that the louse is not the normal insect vector of *T. lewisi* and that it is doubtful whether true cyclical development can occur in that insect. Using a strain of *T. brucei* isolated from game, Duke attempted to make the strain drug-resistant by giving increasing doses of atoxyl. After cyclical transmission through *G. palpalis*, the strain was still atoxyl-resistant. He found that the acquired character of resistance of a trypanosome to arsenic persists after cyclical passage of the strain through tsetse, and that it can survive to at least the third generation of cyclical passage. Unfortunately these results were not altogether conclusive, owing to the fact that the strain of *T. brucei* used had an unsuspected natural resistance to atoxyl.

Schilling and Schreck (1930) examined the effect of cyclical passage through *Glossina morsitans* on an old laboratory strain of *T. brucei*. This old strain was very virulent and differed markedly from a less virulent strain which had been recently isolated from game and had been kept going in experimental animals by a series of fly transmissions. They found that the passage of the laboratory strain through tsetse produced a marked change in its character. The virulence had greatly diminished and the changed strain was now identical both in virulence and serological properties with the 'genuine' strain which had never suffered direct transmission.

EXPERIMENTAL

The object of this work was to determine whether an acquired resistance to human serum can be preserved during the cyclical passage of the game trypanosome through tsetse. For this purpose a strain of *T. brucei*, sensitive to serum, was passed into a series of mice which were given repeated doses of normal human serum. When once the strain had become serum-fast, it was passed by cyclical transmission through *G. tachinoides*. After cyclical passage, the reaction of the strain to human serum was tested again by giving measured doses to infected mice.

CHARACTERISTICS OF ORIGINAL STRAIN

The strain of *T. brucei* used was isolated from wild *G. morsitans* in May, 1930. At the start of these experiments it was five months old, having been passed by direct transmission through a series of three dogs and three rhesus monkeys.

When freshly isolated, the strain was somewhat avirulent, but repeated direct transmissions had exalted its virulence to such an extent that it killed small laboratory animals, guinea-pigs, rats and mice, in a few days. When measured doses of 500,000 trypanosomes were given to a series of white mice, the incubation period was found to be one to two days and the life of a mouse six to eight days. From the moment that parasites appeared in the blood, they increased in numbers steadily until the death of the host. The behaviour of the controls in Table I illustrates this point.

The intraperitoneal injection of 1 c.c. of human serum cleared the peripheral blood of infected mice in about eight hours. The blood remained negative for five days, a scanty infection appearing on the sixth day. Table I shows the result of such an experiment.

PROCEDURE FOR MAKING STRAIN SERUM-FAST

Although numbers of mice were treated with human serum in the endeavour to make the strain serum-fast, it is only necessary to trace the strain through that series which was used eventually for the fly transmission. In all cases the serum was given by the intraperitoneal route.

Mouse 2 was given in all four 1 c.c. doses of serum. After the first dose trypanosomes reappeared in six days, after the second in five days, after the third in four days, and at the fourth dose they failed to disappear. The infection was then passed on into another mouse, which was given two doses of serum. In this animal the trypanosomes were comparatively insensitive, the injection of serum causing some diminution in their number but failing to clear the blood completely. On passing the strain into a third mouse it was found that the trypanosomes had become completely resistant to 1 c.c. of human serum. At this stage the strain had been subjected to a total of seven doses and appeared to have become serum-fast. It was apparent that the serum-fast property was preserved through direct passage from mouse to mouse.

TABLE I
Showing the trypanocidal action of human serum on a strain of *T. brucei* in mice

Animal	Start	4 hours	8 hours	24 hours	2 days	3 days	4	5	6	7
Mouse 1 (given 1 c.c. of serum)	+++ (258 × 10 ⁶)	+++ (156 × 10 ⁶)	—	—	—	—	—	—	+	+
5	++	++	—	—	—	—	—	—	+	+
(H.M.O.L.)	(48 × 10 ⁶)	(96 × 10 ⁶)	—	—	—	—	—	—	+	+
2	++	++	—	—	—	—	—	—	+	+
	(52 × 10 ⁶)	(46 × 10 ⁶)	—	—	—	—	—	—	+	+
4	++	++	++	+++	+++	+++	+++ (died)	—	—	—
	(50 × 10 ⁶)	(62 × 10 ⁶)	(84 × 10 ⁶)	(182 × 10 ⁶)	(182 × 10 ⁶)	(744 × 10 ⁶)	+++ (died)	—	—	—
Controls ...	++	++	++	++	++	++	++	+++	+++	died
3	(20 × 10 ⁶)	(36 × 10 ⁶)	(98 × 10 ⁶)	(282 × 10 ⁶)	++	++	++	+++	+++	+++

NOTE: The numbers in brackets indicate the number of trypanosomes per c.c. of blood.

As the mouse, owing to its size, is not a convenient animal for use in fly transmissions, it was necessary to ensure that the serum-fast property could be preserved after direct passage through guinea-pig. The resistant strain was passed into a guinea-pig which was subsequently given an intraperitoneal injection of 6 c.c. of serum. This dose cleared the peripheral blood of the guinea-pig in a few hours, and on subsequent passage into a mouse the strain was found to have lost a considerable portion of its serum-resistance. It was obvious that further treatment would be necessary before the strain became sufficiently serum-fast for the fly transmission to be attempted.

The resistant strain was passed through a series of mice—guinea-pig—mice, the mice receiving injections of serum. The stage was soon reached at which the trypanosomes were fast to 1 c.c. of human serum, even after intermediate passage through a guinea-pig. Details of these transmissions are given in the accompanying diagram.

FLY TRANSMISSION

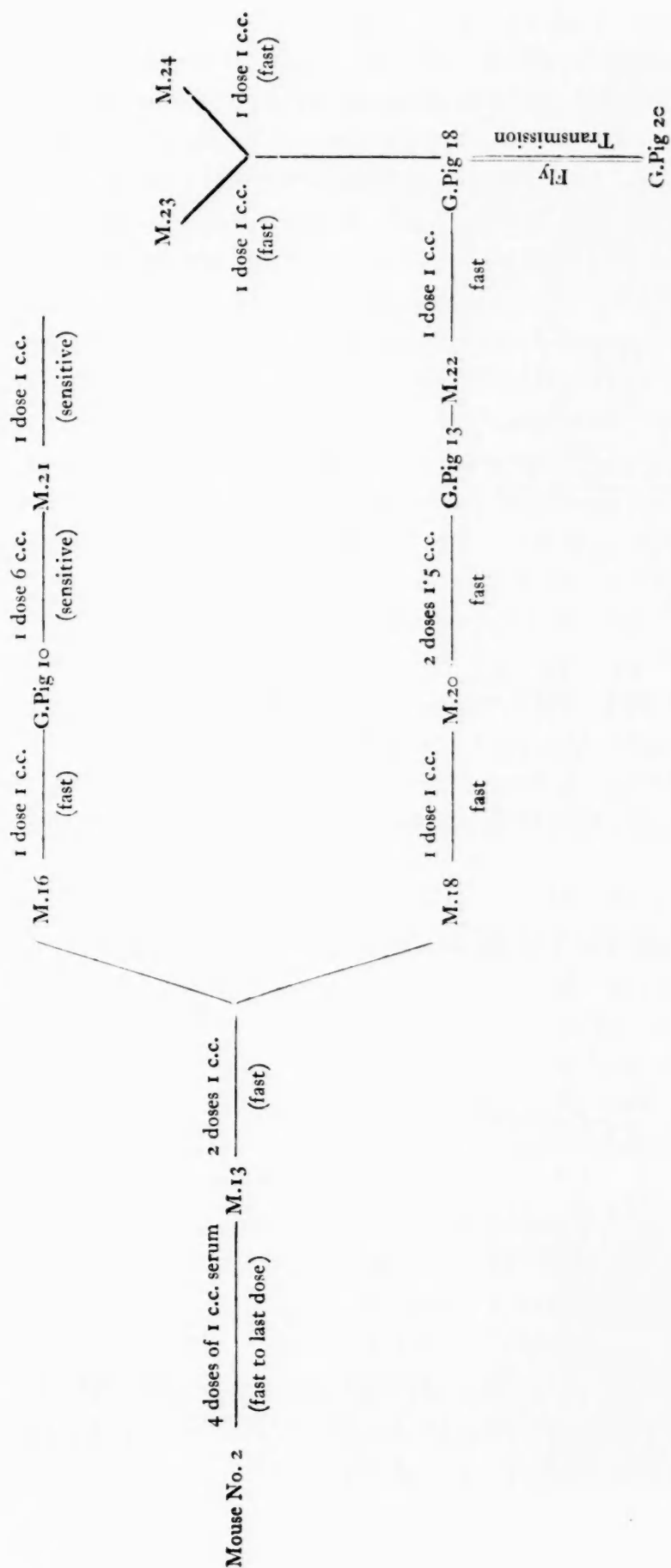
A series of 300 laboratory bred *G. tachinoides* were fed on infected Guinea-pig 18 for six consecutive days, after which they were fed on a clean guinea-pig. A scanty infection showed up in the clean guinea-pig forty-five days after the flies' first infective feed. Other fly transmissions were attempted, but these all gave negative results.

CHARACTERISTICS OF STRAIN AFTER FLY TRANSMISSION

The first thing to be noticed was that cyclical transmission had diminished very greatly the virulence of the strain to small laboratory animals. With guinea-pigs the incubation period was now about six to eight days and the life of a guinea-pig several months. Before cyclical transmission the incubation period in a guinea-pig was about 24 hours and the life of a pig six to fourteen days. The change in virulence to white mice was even more marked. It has already been mentioned that the original strain would kill a mouse in six to eight days, and that with a measured dose of 500,000 trypanosomes the incubation period was one to two days. After the fly transmission

DIAGRAM.

Giving details of transmissions carried out in making strain serum-fast.



it was very difficult to get trypanosomes sufficiently numerous in the peripheral blood to test the action of serum.

A series of white mice were given measured doses of 500,000 trypanosomes. The incubation period was now eleven to fifteen days. These mice lived several months, they rarely showed more than a scanty infection of the peripheral blood, and there were numerous intervals of ten days or more when no trypanosomes could be seen. Even when doses of 20 million trypanosomes were given, the incubation periods remained excessively long, the mice surviving several months and showing numerous relapses. To show that it was not the repeated doses of human serum which had caused this change in pathogenicity, a series of mice were given small doses of trypanosomes from a mouse infected with the resistant strain, which had been kept going by direct transmission and which had not passed through *Glossina*. In these animals trypanosomes increased uniformly in the blood until the death of the host.

To test the reaction of human serum after fly transmission, infected mice were given 1 c.c. doses of normal human serum. In no case were the trypanosomes in the blood sufficiently numerous for numerical counts to be made. Table II gives the results of some of these experiments, the + sign only indicating that trypanosomes were present in the peripheral blood.

TABLE II

Showing the effect of human serum on the resistant strain after cyclical passage through tsetse.

Animal		Start	4 hours	8 hours	12 hours	24 hours	48 hours
Mouse 32	1 c.c. of human serum (H.M.O.L.) on 18.4.31	+	+	+	+	+	-
Mouse 31	1 c.c. of serum (H.M.O.L.) on 25.4.31	+	+	+	+	+	+
" 34		+	+	+	+	+	+
" 36		+	+	+	+	+	+

The dose of serum did not affect the numbers of trypanosomes in the peripheral blood, whereas with the original strain trypanosomes disappeared within eight hours.

DISCUSSION

It has been shown that an acquired resistance to human serum can be preserved through at least one cyclical passage through tsetse. This is a matter of fundamental importance, and there seems no obvious reason why this character, having survived one passage, should not be retained during a number of cyclical transmissions.

Other cyclical transmissions were not successful owing to an unforeseen difficulty in passing the strain through *Glossina*. When first isolated the strain was readily transmissible by tsetse, but later it became very difficult to secure cyclical transmission. In this connection it might be mentioned that a similar difficulty was met with when working with a strain of *T. gambiense* which had been subjected to a considerable amount of manipulation. This strain, originally very sensitive to tryparsamide, was subjected in guinea-pigs to increasing amounts of the drug. After some months the trypanosomes became completely resistant to therapeutic doses of tryparsamide, but all attempts at cyclical transmission through *G. tachinoides* failed. In all, over 1,200 bred flies were used in these failures, though at the start the original strain was successfully transmitted at the first attempt. It is possible that in both these cases the technique used in making the strains fast, had an adverse effect on their transmissibility.

The fact that, before the strain had become completely serum-fast, a direct passage through a guinea-pig caused the trypanosome to lose its resistance to serum is interesting. Yorke and his collaborators postulate that *T. rhodesiense* loses its serum-resistance when it is passed into game or domestic animals instead of man. In this instance the property of serum-resistance was kept during passage from mouse to mouse, but was lost when the strain was passed through the guinea-pig.

The effect of cyclical transmission on the pathogenicity of the strain was very well marked. When first isolated from wild tsetse the strain appeared rather avirulent, but after a number of syringe passages it became very virulent to small laboratory animals. After cyclical transmission the pathogenicity was greatly reduced and if anything was even less than when first isolated. These observations agree in this particular with the findings of Schilling

and Schreck. On the other hand these workers found that the serological properties of their strain were altered by fly transmission. In our instance the particular acquired serological property under consideration was kept. Duke (1928) wrote: 'The experiments . . . on the transmission of arsenic-fast strains, inconclusive though they are, have shaken my faith in the stabilising effect of cyclical passages through fly.' The fact that the serum-fast property could be kept during fly transmission lends some support to Duke's contention that the acquired character of arsenic-resistance persists after cyclical passage. At the same time there is no doubt that cyclical passage through tsetse has a well marked stabilising effect on the pathogenicity of a trypanosome.

This stabilising effect of cyclical transmissions leads us to a matter of some importance, namely, the differences which occur between laboratory strains and the strains as they occur in nature. The greater part of the work on the pathogenic trypanosomes has been done with old laboratory strains kept going for years in small animals. Being passed on directly from one animal to another, trypanosomes are always subjected to the same environment and never have to undergo the changes which take place in the insect host. In the course of time they tend to devote all their energy to rapid multiplication, and their virulence to small animals becomes very much intensified. Eventually the characteristics of a strain may alter to such an extent that it bears little resemblance to the original strain freshly isolated from nature.

Working in the Gadau laboratories, we usually find that it is quite impossible to do anything with strains freshly isolated from human sleeping sickness cases. Trypanosomes in the peripheral blood of laboratory animals are very scanty, and during relapses the blood may remain negative for weeks at a time. With local strains of *T. gambiense* it is only after several months, during which time the virulence has been intensified by a number of syringe passages, that trypanosomes become sufficiently numerous to be of use. The doubt arises as to whether observations made on artificially virulent strains can have much value as an indication of the characteristics of these strains as they occur naturally. Cyclical passage through tsetse causes a strain to revert back to its original pathogenicity, but it has now become too avirulent for laboratory work.

The observations relating to the pathogenicity to mice of the strain of *T. brucei* before and after fly transmission afford a good example of this point. According to the classical description, the behaviour of pathogenic trypanosomes in mice is quite characteristic. It has been the subject of a good deal of work ; the earlier workers, e.g., Massaglia (1907), noted that the parasites appear in the blood after a short incubation period and increase in numbers steadily and uniformly until the death of the animal. Doerr and Berger (1922) found that infections of *T. brucei* increase according to a geometrical progression series. W. H. and L. G. Taliaferro (1922), working with *T. rhodesiense*, observed that reproduction occurs at an approximately constant rate, and that the number of trypanosomes in the blood increases according to a geometrical progression. They conclude from this that no resistance whatever is acquired by the mouse, either affecting the rate of reproduction or destroying trypanosomes after they are formed.

Before fly transmission the behaviour of our strain of *T. brucei* in mice was quite in accordance with these findings. But after cyclical transmission the whole picture was altered. This same strain of *T. brucei* was avirulent to mice, there was a long incubation period and numerous relapses. There certainly was no question of a steady and uniform increase in numbers until the death of the host. In fact, in more than one case the animal lived over six months without ever showing more than scanty infection in the blood. It appears then that *T. brucei* freshly isolated from fly may be very avirulent to mice. Obviously, mice must have some form of defensive mechanism, otherwise one cannot account for the numerous relapses which took place.

These observations possibly throw light on one of the causes of the confliction of evidence which so often occurs in work on the various trypanosomes. It seems by no means improbable that much work done with old laboratory strains does not give a true picture of what occurs in nature.

SUMMARY

1. As a result of their discovery of the trypanocidal action, *in vitro*, of normal human serum, Yorke, Adams and Murgatroyd elaborated a hypothesis regarding the epidemiology of *rhodesiense*

and gambiense sleeping sickness. They postulate that, although *T. rhodesiense* is identical with *T. brucei*, normal human beings cannot be infected with the game trypanosomes because of the trypanocidal power of their serum. In certain pathogenical conditions the blood of man loses its trypanocidal power, allowing the game trypanosomes to become established.

2. If this hypothesis is correct, the serum-fast character, once it has been acquired by the game trypanosomes, must be preserved during cyclical transmission by tsetse. In order to throw some light on this point, a strain of *T. brucei*, originally quite serum-sensitive, was kept in mice which were treated with repeated doses of normal human serum. When once the strain became serum-fast it was passed by cyclical transmission through *G. tachinoides*. On testing again the reaction of the strain to human serum, it was found that the acquired serum-resistance had been preserved through the fly.

3. After cyclical transmission the virulence of the strain to small laboratory animals had diminished very greatly. This was particularly well shown in mice. Before cyclical transmission, the behaviour of the strain was in accordance with the classical description. Trypanosomes appeared in the blood after a short incubation period, and increased in number steadily until the death of the mouse. After passing through tsetse there was a long incubation period, trypanosomes were never at all numerous in the blood, and there were numerous relapses. Several mice lived more than six months, showing only an occasional scanty infection in the blood. In view of these observations it is difficult to believe that the mouse can have no defensive mechanism against the pathogenic trypanosomes.

4. The doubt arises as to whether much of the work done with old laboratory strains, the characteristics of which have been changed by constant syringe passage, can give a true picture of what occurs in nature.

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COCCIDIOSIS OF SKUNKS

BY

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PLATE II

At the present time, in the family of *Mustelidae*, are found the following coccidia : (1) *Eimeria ictidea* Hoare, 1927, in African fitchets (ferret); (2) *Eimeria furonis* Hoare, 1927, in the same animal; (3) *Isospora laidlawi* Hoare, 1927, in the same; and (4) *Eimeria mephitidis* Andrews, 1928, in the American skunk (*Mephitis mephitis*).

At the beginning of this year (1932), we found in the skunk (*Mephitis hudsonica* Richardson) a coccidium belonging to the family of *Eimeria*. Faeces of skunks were sent to us by Mr. Poljansky from the skunks of a farm of racoons in Woronesch. There were, in all, faeces of 13 animals, and coccidia were found in 10 (76.9 per cent.).

The shapes of the oöcysts after Darling were round, nearly round, oval and, very seldom, oviform. The membrane had two contours, with sometimes apparently three. There was no micropyle. The protoplasm was tightened into a thick granulated ball. The size is shown in Tables I and II.

These tables indicate the width of the oöcysts :—

(1) *Oval*, *oviform* or *almost round* (measured 57 oöcysts) : 17.5 to 27.0 μ \times 15.0 to 23.4 μ , average 23.1 μ \times 20.5 μ . The greatest size 27.0 μ \times 23.4 μ ; the smallest 17.5 μ \times 15.0 μ ; the most frequent ('typical size') 23.4 μ \times 21.6 μ . Form index 1 : 0.73 to 0.98; average 1 : 0.92; the most frequent ('typical form index') 1 : 0.92.

TABLE I
Oval and oviform.

	17.5	18.0	18.5	19.0	19.5	20.0	21.0	21.6	22.0	22.5	23.0	23.4	25.0	25.2	26.0	27.0	Total
15.0	I (0.85)	I (0.78)	...	I (0.75)	3
16.0	...	I (0.88)	1
16.5	I (0.89)	1
17.5	I (0.83)	1
18.0	4 (0.85)	4
18.5	I (0.94)	I (0.92)	2
19.0	I (0.90)	1
19.5	I (0.80)	1
19.8	I (0.94)	3 (0.91)	3 (0.84)	...	2 (0.78)	...	I (0.73)	10
20.0	I (0.98)	...	I (0.90)	2 (0.88)	I (0.80)	5
20.7	I (0.92)	...	I (0.88)	...	3 (0.82)	5
21.0	I (0.95)	...	I (0.91)	...	I (0.84)	I (0.83)	4
21.6	6 (0.92)	...	4 (0.85)	10
22.5	I (0.86)	...	1
23.4	6 (0.92)	...	2 (0.86)	8
Total	I	I	I	I	I	2	8	3	2	3	2	10	2	16	I	3	57

TABLE II
Round

$\mu\mu$	18.0	19.8	21.6	23.4	24.3	25.2		21.8
			I	7	23	6	I	3	41	Average

(2) *Round* (measured 41 oöcysts): 18.0μ to 25.2μ ; average 21.8μ ; frequently occurs ('typical size') 21.6μ .

We cultivated the faeces in a solution of 2.5 per cent. of potassium bichromate. Each oöcyst developed four sporoblasts (size 10.8μ), producing spores of an oval form with rounded or sharp ends, size 9.3 to $11.7\mu \times 7.2$ to 7.7μ . In the spores two sporozoites were developed, pear-shaped and bent, size 6.5 to $7.0\mu \times 2.0$ to 2.7μ . Residual bodies are present only in the sporocysts, the oöcysts having none.

We fed a whelp with entirely developed oöcysts. The faeces of the whelp had been several times looked over, but no coccidia were seen. The first day, the faeces contained some rather undeveloped oöcysts. During the next 6 days nothing was found in the faeces. Some round oöcysts (size 18.0 to 19.8μ) and some of an oval shape (size 19.8 to $21.6\mu \times 18.0$ to 19.8μ) appeared in the faeces on the 9th day. On the 10th day, there were observed round and oval shapes of the same size, and also on the 12th day. On the 13th day, there appeared only oval shapes of the following size (Table III).

TABLE III

	19.8	21.6	22.5	23.4	Totals
16.2	...	¹ (0.74)	1
18.0	³ (0.90)	⁴ (0.83)	7
19.8	...	¹³ (0.91)	¹ (0.88)	⁴ (0.84)	18
21.6	¹ (0.92)	1
Total ...	3	18	1	5	27

We can see from this table that the size is as follows:— 19.8 to $23.4\mu \times 16.2$ to 21.6μ ; average $22.9\mu \times 19.2\mu$; the greatest $23.4\mu \times 21.6\mu$; the smallest $19.8\mu \times 18.0\mu$; frequent ('typical size') $21.6\mu \times 19.8\mu$. Form index $1:0.74$ to 0.92 ; average $1:0.88$; most frequent ('typical form index') $1:0.91$. We can

see that between these sizes and the above-mentioned there is but little difference (Table I).

Afterwards, beginning from the 14th day after feeding, the oöcysts disappeared and did not again occur in the faeces. This means that the whelp underwent a short-timed infection with this coccidium, that lasted only five days.

The oöcysts of the whelp sporulated in a solution of 2.5 per cent. of potassium bichromate after 24 hours.

We are comparing our *Eimeria* with those at present known in animals of the family *Mustelidae* (Table IV).

TABLE IV

Species	Animals	Shape	Size of oöcysts		Size of spores		Residual body	
							in oöcysts	in sporocysts
<i>Eimeria furonis</i> Hoare, 1927 ...	Ferret	Round	Average 18.8	12.0	8.5	4.0	○	+
<i>Eimeria ictidea</i> Hoare, 1927 ...	Ferret	Round	Average 23.5	17.5	11.5	6.5	○	+
<i>Eimeria mephitidis</i> Andrews, 1928	Skunk (<i>Mephitis mephitis</i>)	Oval	17-25	16-22	11	8	○	+
Our <i>Eimeria</i> ...	Skunk (<i>Mephitis hudsonica</i>)	Oval, Oviform, Round	17.5-27.0 18.0-25.9	15.0-23.4	9.3-11.7	7.2-7.7	○	+

We assume that the coccidium of the skunk (*Mephitis hudsonica* Richardson) found by us is the same as Andrews (1928) discovered in the United States of America, i.e., *Eimeria mephitidis*.

One ought to notice the very interesting fact that this coccidium produces a short-timed infection in whelps.

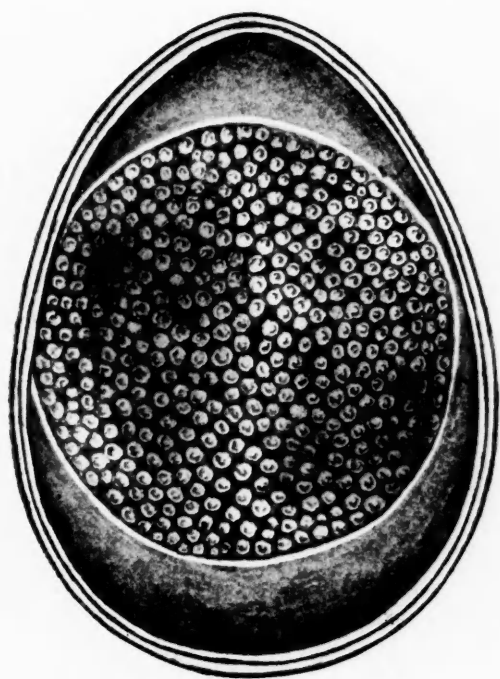
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PLATE II

EXPLANATION OF PLATE II

Eimeria mephitidis Andrews, 1928, of Skunks (*Mephitis hudsonica*).



Dr. Perecropoff, del.



AN EXPERIMENT FOR IMMUNIZATION AGAINST CUTANEOUS LEISHMANIASIS

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(Received for publication 17 September, 1932)

INTRODUCTION

Up to the present, immunization against cutaneous leishmaniasis has been attempted in Iraq only by means of active immunity produced by inoculations of human sore into the skin in such places where the resulting scar would be of no importance. The indigenous population, especially the Jewish inhabitants of Baghdad City, sometimes use this type of artificial inoculation from naturally occurring cutaneous lesion, but it leads accidentally to very serious generalized infection.

An interesting method of successful inoculation in a voluntarily chosen area was related to the writer by a telegraph official from Fao, in the Persian Gulf, who entered Mesopotamia by the way of Aleppo-Deir Zor in December, 1905. A young man of a party of five, M. N., from Constantinople, was advised to rub his foot or hand with some earth, picked up immediately on his entrance to Mesopotamia, in order to avoid contracting oriental sore on the face. He followed this advice in Aleppo, rubbing his right foot with sand, to the great amusement of his four companions. They continued their way to Basra and subsequently to Fao, which they reached in the first days of January, 1906. The incident was forgotten. But in February, a dermal lesion developed exactly on the spot of the supposed inoculation on the right foot of M. N. During the next summer three members of the party contracted cutaneous leishmaniasis on various parts of the body.

It is of interest to note that the date of the uncommon process of inoculation did not correspond with the period of natural

contamination, the occurrence of cutaneous leishmaniasis showing in Iraq a very definite seasonal incidence.

In view of the benign character of oriental sore, two factors only have to be considered as a reason for any attempt of immunization against it—(1) the possibility of localization in the face; (2) the annoyance of the dermal lesion lasting several months.

Infants are especially apt to contract oriental sore on the face, and their lesions result invariably in some kind of cosmetic defect. The scar is not of equal importance in adults, their infection being usually localized on the exposed parts of the extremities.

The period of duration of cutaneous leishmaniasis can be much shortened with a radical and early treatment; but the earlier we proceed against it, the less probable is a complete development of active immunity, which seems to be in direct proportion with the length of duration of the sore. This is a point which, up to the present, made vaccination against cutaneous leishmaniasis a difficult problem. Parrot's experiments led to the following conclusion: 'Pour obtenir l'immunité contre la leishmaniose cutanée, l'infection réelle du sujet est nécessaire. Un vaccin antileishmanien ne vaccinera que dans la mesure où il aura produit une réaction manifeste.'

The writer, who lived in central quarters of Baghdad City, Haidar khaneh and Ras el Qariyah, where the infection is prevalent, contracted cutaneous leishmaniasis during the first summer, and from September, 1925, to December, 1925, developed 12 papules on various parts of her arms and legs, as well as on her face. Ten of them were treated by electrocoagulation, a method which the writer considers more convenient and simple for the treatment of cutaneous leishmaniasis than other methods actually in use. The remaining sores were allowed to take a natural course for the purpose of future immunity.

DESCRIPTION

It occurred to the writer that it would be of interest to try a new method of immunization against cutaneous leishmaniasis on the basis of Besredka's theory of local immunity. It seemed probable that a skin graft from a person enjoying active immunity

against oriental sore could transmit the specific qualities of its tissue to another person whose skin it would heal in a similar way as foreign immune blood-serum causes the production of immunity in the blood medium.

The first transplantation of skin for this purpose was performed on August 26th, 1927, in the First Surgical Clinic, University Charles IV, Prague, Czechoslovakia. A dermal graft, 10 mm. \times 2 mm., cut from the direct vicinity of the scar of an oriental sore on the writer's leg, was implanted into a corresponding incision in the arm of her Czechoslovak nurse, M. M., 23 years old, who was proceeding shortly to Baghdad, to take up service there for the first time. The graft healed in its full length. The nurse arrived in Baghdad at the end of September, 1927, and remained there until the middle of May, 1930, without contracting oriental sore, although the surroundings of her residence were infected, and other Europeans in the first summer of their arrival contracted cutaneous leishmaniasis immediately.

This experiment was repeated by the writer in 11 more cases in children and infants. The skin graft had been obtained from one of the children's parents, who had infection with oriental sore in their history. The transplantation was always performed under local anaesthesia, and, after the graft had been well adapted into the incision, collodion, poured over the wound, was used as the only dressing. Gauze with a layer of sterile vaseline, used as a dressing in skin grafting, proved unsatisfactory.

With regard to the areas from which the grafts had been removed, the cases which gave satisfactory results had been implanted with skin grafts removed from the immediate vicinity of a scar of oriental sore. In other cases, where the only scar was on the face, the grafts were taken from intact parts of the extremities.

HISTORY OF INFANTILE CASES

Three cases were lost sight of after transplantation, and consequently no further observation was possible.

The skin graft of another case was found necrotic under the gauze-vaseline dressing.

Another graft had been washed out through bleeding, due to excessive restlessness in the child.

Below is the history of cases where the graft healed normally and where permanent control was possible.

CASE 1. Radbor D. L., the writer's son, born on July 28th, 1928.

14.II.28. Under local anaesthesia with $\frac{1}{2}$ per cent. novocain-adrenalin, was transplanted to him, in an incision in the right parietal region, a skin graft of 8×1 mm., removed from the vicinity of the scar of an oriental sore from his mother's knee, two years after healing of the sore. Dressing with collodion.

The transplantation was performed in this case before any other immunizing intervention, in order that its effect on the vaccination against variola might be observed. The mother had last been revaccinated in the summer of 1927, with negative result.

21.II.28. Vaccination against variola with a lymph of Swiss origin. Negative result.

29.II.28. Collodion removed. More than 3 mm. of the graft healed well, the remaining part sticking dry to the collodion.

29.II.28. Revaccination with fresh lymph from the Vaccine Lymph Institute, Baghdad. Negative result.

27.4.29. Second revaccination with high potency lymph (98 per cent.) from the Vaccine Lymph Institute, Baghdad. Positive result.

The child lived in Ras el Qariyah, Baghdad City, until the spring of 1932, without contracting cutaneous leishmaniasis.

CASE 2. Doris M., 2 months old, Jew. Mother, 29 years old, born and living in Baghdad, contracted cutaneous leishmaniasis on the face in early childhood.

19.8.29. Transplanted into the child's parietal region a skin graft of 9×1 mm. removed from the mother's leg.

5.9.29. The graft healed in its full length.

22.9.31. The child lived from autumn, 1929, to summer, 1930, in Hillah on the Euphrates, and returned to Baghdad in the autumn of 1930. In September, 1931, she developed two dermal lesions on the face, showing the typical papular characteristics of cutaneous leishmaniasis.

CASE 3. Shehim F., 5 years old, born in Smyrna, arrived in Baghdad in December, 1928. Father, 40 years old, born in Baghdad, was infected with cutaneous leishmaniasis in his childhood; mother, born in Smyrna, contracted oriental sore in Baghdad six years before. Residing in Dukan Shenawi quarters, Baghdad.

26.2.29. Owing to the refusal of the child's mother to supply the skin graft, a graft was removed from the father's arm and transplanted in the usual way into the arm of the child.

The boy left Baghdad in August, 1929, for Constantinople. It is stated that he developed an oriental sore in the autumn of the same year.

CASE 4. Mater M., 3 months old, Arab, living in Karradet el Mariam, Baghdad West. Mother, 36 years old, born in Baghdad, infected with cutaneous leishmaniasis in her childhood, with several scars on her face.

12.4.30. Transplantation of a skin graft, removed from the mother's arm, into the arm of the child.

23.9.30. Examination revealed three papular lesions on the nose and cheeks, with typical characteristics of cutaneous leishmaniasis.

CASE 5. Maisun E. A., 1 year old, Arab, born in Damascus, Syria. Arrived in Baghdad in December, 1930. Mother, 34 years old, from Damascus, was infected, together with her two sons, on her first visit to Baghdad in 1926, with cutaneous leishmaniasis, which caused to all three considerable cosmetic defects. She consented to bring her daughter to Iraq, on condition that there were means for preventing the infection of oriental sore.

24.2.31. Transplantation of a skin graft from the mother's arm into the left arm of the baby girl.

Until the end of observations in the spring of 1932, the child remained free from cutaneous leishmaniasis, although the family lived in the same quarters, Baghdad West, as in 1926.

CASE 6. Lydia D.L., born in Baghdad on Jan. 26th, 1931, writer's daughter.

5.3.31. Transplantation of a skin graft from her mother's leg, from the immediate vicinity of a scar of an oriental sore healed six years earlier.

4.4.31. Vaccination against variola with a fresh lymph from the Vaccine Lymph Institute, Baghdad. Negative result.

6.6.31. Revaccinated with a lymph of high potency (100 per cent.) from the same Institute. Positive result.

The child was exposed to one season of contamination, living in Ras el Qariyah, in the centre of Baghdad City, until the end of March, 1932, when she left Iraq without contracting cutaneous leishmaniasis.

SUMMARY AND CONCLUSIONS

The writer adopted a new method of passive immunization against oriental sore, based on the local immunity of Besredka, and consisting of transplanting a small skin graft, removed from an immune person, into a corresponding incision in the skin of a person to be immunized.

Seven out of 12 of her cases, all living in those districts of Baghdad where practically no one escapes the disease, had been observed from 1 to 4 years after the transplantation. Out of these, 4 remained free from cutaneous leishmaniasis and 3 contracted oriental sore during the first season of contamination.

The cases of the first group, in which the result of this experiment was satisfactory, were implanted with grafts removed from intact skin in the vicinity of a scar of an oriental sore 1, 2, 5 and 6 years old. It is evident that the suppliers of the skin grafts for this group,

one of whom was the writer herself, were still enjoying comparatively fresh active immunity.

In the cases of the second group, in which the method failed, the grafts were obtained from persons of 29, 36 and 40 years of age, born in Baghdad and infected with oriental sore in their early childhood. Their immunity proved to be fading away or too weak to be transmitted to a second person.

There is at present an absolute lack of any other reliable means of immunization against cutaneous leishmaniasis. Therefore the method introduced by the writer may be useful under suitable conditions, and may be of real value especially to European families whose children are exposed to contamination in centres of endemic oriental sore.

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A NOTE ON THE RELATIVE SIZE OF THE ANAL GILLS OF MOSQUITO LARVAE BREEDING IN SALT AND FRESH WATER

BY

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Dr. F. W. Edwards (1921), in his 'Revision of the Mosquitos of the Palaearctic Region,' when dealing with *Aedes* (*Ochlerotatus*) *capsius* Pallas, mentions that the anal gills of larvae from different regions differ in size. He finds that some show a reduction commonly seen in species from salt and brackish water, and suggests that the longer gilled specimens may have come from fresher water. The observations of the writer carried out during November and December, 1931, in the Toro and Busongoro districts of Uganda, show clearly that this is the case in some species of Ethiopian mosquitoes.

The anal gills of four species of mosquitoes—*Anopheles christyi* N & C., *Culex vansomereni* Edw., *Culex pipiens* L. and *Culex decens* Theo.—found breeding in both salt and fresh water have been studied, and are figured below. They are all drawn to the same scale, and show the gills of the salt water larvae alongside those of the same species from fresh water. The determinations of the larvae were confirmed by rearing adults and, in the case of the culicines, by examination of the male terminalia.

The larvae of *Anopheles christyi*, *Culex vansomereni* and *Culex pipiens*, with the shortened anal gills, were taken from a salt-lick situated in forest near Fort Portal and used by big game. At the time of the writer's visit, the salt-lick was a mire covered with a thin film of brackish water (pH. 9.5); the larvae of *Anopheles christyi* were very numerous, and when disturbed appeared to skate across the surface seeking refuge some distance away. Those with the

normal gills were obtained from a turbid rain-water pool. The short-gilled larvae of *Culex decens* were found in a backwater (pH. 10.5) of the Salt Lake of Katwe, and the normal specimens were taken from the edge of swamp near Fort Portal. In Lake Katwe itself, which is supersaturated with salt, no mosquito larvae were obtained, but the highly concentrated salt water of the backwater had become to a great extent diluted by fresh water from a spring in the hillside. However, the water was still decidedly salty to the palate.

The anal gills of all the larvae from salt water show a reduction in length, and in the case of some species the organ has assumed quite a different shape. In the case of *Anopheles christyi* and *Culex decens* the relative difference is very striking. The fresh water gills of *christyi* (fig. 1) are long, with almost parallel sides and rounded

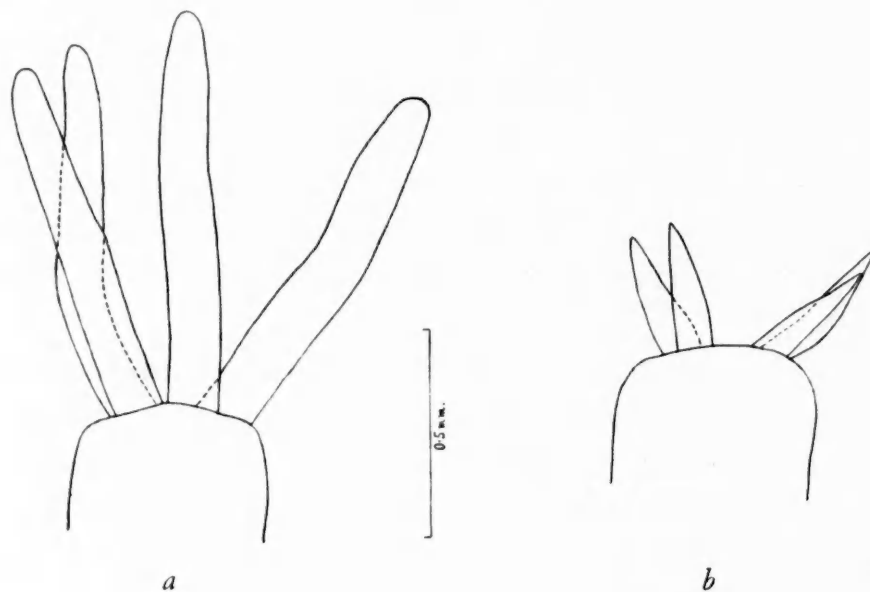


FIG. 1

ends, whereas those from salt water are about a quarter of the length of those from fresh water, and taper from the base to the tip. The larvae of *decens* (fig. 2) from saline water have short, stubby gills, while those from fresh water are long and taper to a point. The dissimilarity of the salt and fresh water gills of *Culex vansomereni* (fig. 3) is not so marked, though those from saline water are distinctly the shorter. The larvae of *Culex pipiens* (fig. 4) found in brackish

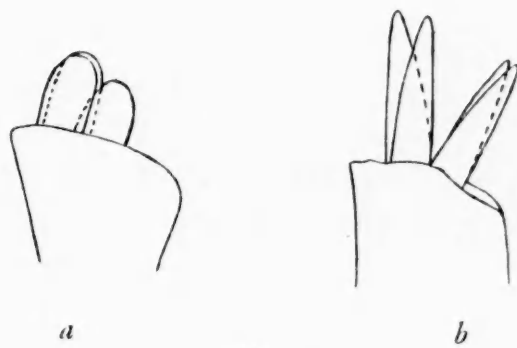


FIG. 2

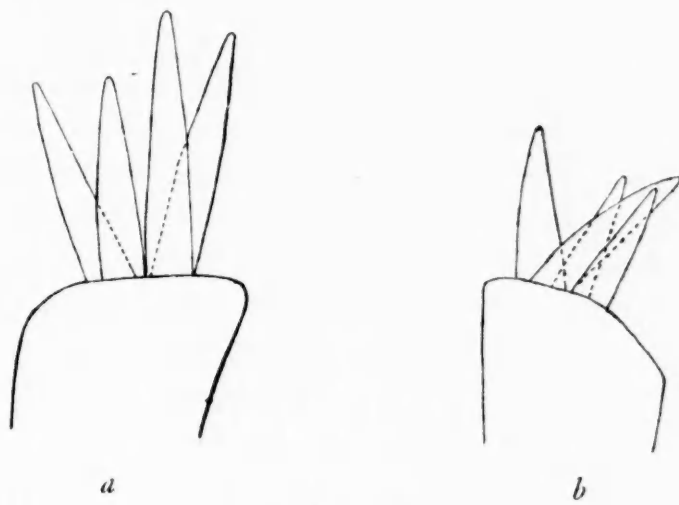


FIG. 3

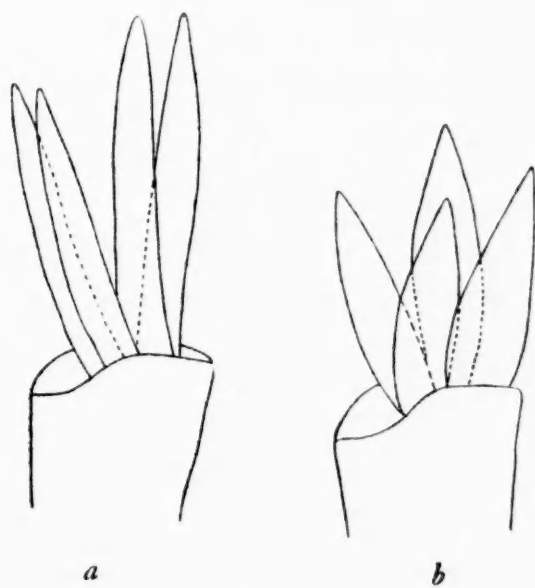


FIG. 4

water have broad and lanceolate gills, whereas those from fresh water are slender and taper to a blunt tip.

The writer is indebted to Dr. A. M. Evans, of the Liverpool School of Tropical Medicine, for kindly directing his attention to the note by Dr. Edwards on the subject, and for confirming his identification of the salt water specimens of *Anopheles christyi*.

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EXPERIMENTS ON PLASMOQUINE AS A PROPHYLACTIC AMONG WEST AFRICAN NEGROES EXPOSED TO BITES OF *A. COSTALIS* INFECTED WITH SUBTERTIAN MALARIA

BY

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These experiments were carried out on the rubber plantations in Liberia, maintained by the Firestone Tire and Rubber Company, from August, 1931, to December, 1931. The object was to test the effect of plasmoquine on the sporozoites of subtertian malaria when injected into human beings by the bites of anopheles infected in the laboratory; in other words, a test of plasmoquine as a prophylactic in the strict sense. We have not attempted to determine whether or not plasmoquine may affect early stages of the parasites immediately subsequent to the sporozoite stage, or whether the action of the drug on the parasite is direct or indirect. Our chief aim has been to determine the amount and character of treatment suitable for the prevention of subtertian malaria.

LITERATURE

Fischer (1927) tested the effect of prophylactic plasmoquine on a ship's crew cruising on the west coast of Africa. The dosage, 0.055 gm. mornings and 0.040 gm. evenings, was given on 3 successive days of the week during a voyage of 9 weeks. Of the persons treated, approximately 37 in number, 15 per cent. suffered from attacks of malignant tertian, all light cases with no formation of

* The author is indebted to Dr. M. A. Barber, of the International Health Division of the Rockefeller Foundation, for initiating this work and making various suggestions during the course of it. Some of the suggestions were sent by wireless through the courtesy of the Firestone Plantations Company, which has wireless stations in Akron, Ohio, and on its plantations in Liberia.

crescents. Unprotected crews of ships which made approximately the same voyage and at the same time gave 25 to 30 per cent. morbidity.

Ottolenghi and Brotzu (1929 and 1930) treated human subjects with small doses of plasmoquine and quinine, and in the course of the treatment caused them to be bitten by mosquitoes infected with benign tertian and malignant tertian malaria. The patients remained free from malaria during the treatment, but many of them came down with malaria after the treatment had been discontinued.

Ronnefeldt (1931) gave 2 European adults a dosage of at least 1 tablet of plasmoquine compound daily and 2 European children the same dose every other day. No harmful effects were observed. The subjects lived in Liberia (Cape Mount) and remained free from malaria at least 1½ years. On return to Europe, however, one adult had an attack of malaria (personal communication).

James *et al.* (1931) protected 10 human beings against sporozoites of benign tertian malaria by a daily dosage of 0.06 gm. of plasmoquine given in three 0.02 gm. doses; while of 4 controls who were taking no medication and one control who was taking quinine, all acquired malaria with parasites in the blood. The same mosquitoes that were used to bite the plasmoquine protected subjects were used on the controls.

Swellengrebel and de Buck (1931) inoculated 13 subjects with benign tertian malaria by the bites of from 5 to 12 infected mosquitoes. On the evening preceding mosquito inoculation, each subject received 0.01 gm. plasmoquine, and on the following morning a second dose of 0.01 gm. plasmoquine. Three hours after this second dose, 9 of the subjects were bitten by the infected mosquitoes. The other 4 subjects were bitten after the third 0.01 gm. dose of plasmoquine. Three doses of 0.01 gm. each of plasmoquine were given each day for 6 days, counting the day of mosquito inoculation. Twelve of these 13 cases, as compared with controls taking no plasmoquine, were not protected against malaria; nor were the attacks of malaria accompanied by the appearance of parasites in the blood delayed. The thirteenth case remained free from malaria for 7½ months, at which time he had a typical attack accompanied by parasites in the blood (Swellengrebel, 1932).

It appears that plasmoquine has a more or less specific effect in

preventing or delaying a primary attack of malaria, possibly by its action on the sporozoites of malaria. The literature does not give any very definite information regarding the proper dosage or the duration of protection, at least in the case of subtertian malaria. The destructive action of plasmoquine on crescents has been proved by the work of many authors.

REQUIREMENTS FOR A GOOD PROPHYLACTIC

In order to be of practical value, a prophylactic must not only have a destructive action on parasites but must be harmless to the patient, even when taken continuously over long periods of time. And, as a matter of convenience, it should be effective when given not oftener than once per day.

Quinine, which is now being taken as a preventive by large numbers of Europeans living in the tropics or other highly malarious districts, does not appear to be a true prophylactic. The action consists in holding the multiplication of the parasites in check, and in this manner lessening the number of attacks of malarial fever, the usual result being that, once quinine is stopped or the individual suffers any diminution of his resistance, the parasites gain the upper hand and an attack of malarial fever ensues. If plasmoquine should prove to be a true prophylactic and an adequate dosage harmless, it should be far superior to quinine as a preventive of malaria.

CONDITIONS UNDER WHICH EXPERIMENT WAS PERFORMED

The fact that this experiment was carried out in Liberia on native West Africans, most of whom harbour malarial parasites for the greater part of their lives, served to complicate an otherwise simple experiment. In a malaria survey conducted in 1931 by M. A. Barber among apparently healthy adults in this locality, 75.1 per cent. of people over 16 years of age were found to harbour parasites; and in another survey by the writer, 82.7 per cent. of all ages were found positive. In all blood examinations done in the course of this work, the Giemsa thick film method was used, and at least 100 fields examined.

Despite this extremely high parasite rate, the malarial morbidity rate is extremely low in adults. Native West Africans carry their parasites well indeed. While it cannot be said that harbouring large numbers of parasites causes no indirect harm to their hosts, still among native West African adults cases of illness or even elevation of temperature due to uncomplicated malaria occur but rarely. These two facts considered together suggest that West Africans acquire a considerable amount of tolerance to the effects of malaria parasites, but the evidence is not so clear regarding their 'anti-parasitic immunity,' since they harbour many, and sometimes vast numbers, of parasites over long periods.

The search for gametocyte carriers with which to inoculate mosquitoes was carried out among the labourers and their families living on the Firestone rubber plantations and other natives living near the plantations. The bloods of 846 people of all ages were examined with the following results:—

Five hundred and forty, or 63.9 per cent. of those examined, had light infections with subtertian schizonts (light infections being less than 1 parasite per thick film field). There were 159, or 18.8 per cent. of the total, heavy infections (that is, 1 or more parasites per field), making a total positive of 82.7 per cent. for all ages. One hundred and thirty-eight showed quartan parasites—16.3 per cent. of the total slides examined. Fifty-four slides showed crescents—6.3 per cent. of the total. Of the 54 crescent carriers, only 6 had a sufficient quantity of gametocytes to warrant their use in a feeding experiment; 4 of the 6 carriers were children. Unfortunately, the best carrier, who had 473 crescents per mm. of blood, was unwilling to be bitten. The 5 remaining carriers were used to infect the mosquitoes.

At the time of biting, a leucocyte count and a thick film blood smear were made of the carrier. The number of gametocytes per 1,000 leucocytes was noted, and the number of gametocytes per c.mm. of blood calculated. In every instance, both male and female gametocytes were observed, but a differential count was not made in all instances.

A total of 3,179 *A. costalis*, the only mosquitoes used in this experiment, was bitten on the five carriers at various times during the experiment, and kept in mosquito cages for 16 days to allow time

for the maximum number of sporozoites to develop. Of the 3,179 mosquitoes fed on the carriers, 442, or 10.8 per cent., remained alive after 16 days. This high mortality is due in part to improper care of one large batch of mosquitoes, nearly all of which died.

Table I shows the number of carrier-fed mosquitoes subsequently dissected, the number found positive for sporozoites in the salivary glands, and the percentage containing sporozoites, together with the approximate number of gametocytes present in the carrier's blood at the time the mosquitoes were fed. Each mosquito had only one meal on the gametocyte carrier.

TABLE I
Gametocyte carriers used in these experiments
Result of test dissections with numbers positive for sporozoites

No. cres. per c.mm. blood	CARRIER No. 1			CARRIER No. 2			CARRIER No. 3			CARRIER No. 4			CARRIER No. 5			TOTAL		
	No. dis.	No. pos.	% pos.	No. dis.	No. pos.	% pos.	No. dis.	No. pos.	% pos.	No. dis.	No. pos.	% pos.	No. dis.	No. pos.	% pos.	No. dis.	No. pos.	% pos.
0-10	30	4	13.3	6	1	16.6	—	—	—	17	4	23.6	—	—	—	53	9	17.0
11-20	—	—	—	4	0	0.0	29	6	20.7	6	1	16.6	49	15	30.6	88	22	25.0
21-30	85	21	24.7	—	—	—	19	6	31.6	—	—	—	—	—	—	104	27	26.0
31-40	54	26	48.1	—	—	—	—	—	—	—	—	—	13	3	23.1	67	29	43.3
41-50	38	12	31.6	5	1	20.0	—	—	—	—	—	—	27	10	37.0	70	23	32.9
51 & over	12	1	8.3	—	—	—	—	—	—	—	—	—	21	1	5.0	33	2	6.1
													TOTAL ...			415	112	27.0

GENERAL PLAN OF EXPERIMENT

The subjects for this experiment comprise 42 native West Africans, all of whom were employed by or lived adjacent to the Firestone Hospital, and 1 European, the writer. The general plan of the experiment was as follows :

The bloods of these 43 people were examined, 26 of whom were found to contain subtertian parasites. Every blood examination throughout the entire experiment consisted of 100 microscopic fields of a thick film preparation stained with Giemsa's stain.

It would have been preferable in an experiment of this nature to use as subjects individuals who had never had any form of malaria. However, it is also important to determine whether or not plasmoquine can prevent re-infection of malaria in individuals who have had malaria previously and have been freed of their parasites in so far as a course of quinine is able to accomplish this.

All of the 43 subjects, regardless of whether their bloods were positive or negative, were given a 2 weeks' preliminary course of approximately 1.0 gram of quinine and 0.02 grams of plasmoquine a day, in order to eliminate as far as possible pre-existing infections.

The subjects were then divided into various groups : the controls, who received no further medication ; and the various sub-groups, consisting of those who were to receive different doses of plasmoquine or other treatment. In the case of individuals who were to continue their plasmoquine while being subjected to the bites of infected mosquitoes, the plasmoquine administration was started immediately after the preliminary course of plasmoquine and quinine, so that there was no interval between the preliminary and subsequent treatments, during which time they might be bitten by wild infected mosquitoes while unprotected by plasmoquine.

During the preliminary plasmoquine-quinine course and up to the time of the first biting by infected mosquitoes, each subject's blood was examined every 3 or 4 days. After the first examination and up to the time of biting by infected mosquitoes, all examinations were negative.

Immediately after the biting by infected mosquitoes, the blood of each subject was examined for malaria parasites, and the examination

continued on alternate days until the subject was discharged from observation. Subjects were discharged from observation (1) when parasites appeared in the blood; (2) when a sufficient interval had elapsed for parasites to have appeared, had inoculation by mosquitoes been successful. A subject was considered 'protected' if no parasites appeared in his blood during the period of observation following attempted inoculation. Subjects were considered 'unprotected' if parasites appeared in the blood any time during the period of observation following inoculation, regardless of whether or not the appearance of parasites was accompanied by an attack of malaria.

All the subjects, with the exception of the one European, were living in unscreened native houses. In making up the various groups, care was taken to insure that no group differed from any other group in the type of food, nature of house, etc., of its individuals. For example, if four individuals were known customarily to enjoy a more nutritious diet or to live in better houses, etc., these four were not placed in one group, but divided up amongst the several groups.

Immediately after the mosquitoes had been fed on the subject to be inoculated, they were killed and dissected, to determine the presence or absence of sporozoites in the salivary glands. The Barber (1930) technique was used throughout the dissections.

GROUP A (Table II), subjects Nos. 1 to 10 inclusive, are controls. Subjects Nos. 1, 2, 4, 5, 8, 9 and 10 had positive bloods at the beginning of the experiment. After their 2 weeks' preliminary course of quinine and plasmoquine, they received no medication whatsoever. The total amount of quinine taken by each subject is shown in the fourth column of the table. During the 2 weeks' preliminary course, each subject received, in addition to the quinine, 0.02 gm. of plasmoquine simplex per day.

Each subject was bitten in the laboratory by the number of infected *A. costalis* noted in the sixth column of Table II. In addition to these known inoculations by infected anophelines in the laboratory, at least a portion of these subjects must have been bitten by wild infected mosquitoes during this period, as no steps were taken to protect them.

Chance wild inoculations do not seriously affect the purpose of this group, however, as the object was merely to show that the

subjects, once freed of their existing infections, could be re-infected with subtertian malaria by the bites of infected mosquitoes.

It will be noted that in the case of subject No. 5, 56 days elapsed between the laboratory inoculation and the finding of parasites in the blood. It seems possible that this subject escaped infection when bitten by an infected mosquito in the laboratory, only to be inoculated by a wild mosquito later.

Also, in subject No. 9, the time between the laboratory bite and the appearance of parasites is so short that the parasites found might have been a relapse from his old infection, and not due to the laboratory inoculation at all.

TABLE II. CONTROLS

Subjects receiving no medication during mosquito inoculation period

Subject no.	Age in years	Weight in kilos.	Total quinine during preliminary course (in gms.)	No. of days between last dose of quinine and mosquito inoculation	No. of infected mosquitoes used	No. of days between biting by infected mosquitoes and finding of parasites in blood
1	22	65.9	14.4	12	1	20
2	30	63.6	10.4	24	4	30
3	24	65.0	16.6	27	1	28
4	18	54.5	16.4	9	1	10
5	30	60.9	16.4	29	1	56
6	17	61.4	11.0	24	4	24
7	16	61.8	11.0	25	3	12
8	19	60.5	11.0	26	3	27
9	17	54.5	11.0	24	2	5
10	18	62.7	11.0	23	3	28

All subjects of this group developed parasites. Since there was no case of illness or even elevation of temperature among them, it would seem that all of them had sufficient tolerance to prevent the appearance of symptoms after mosquito inoculation with subtertian malaria. But since all eventually developed parasites, it would seem that none of them had sufficient 'anti-parasitic immunity' to prevent infection.

GROUP B (Table III), comprising subjects Nos. 11 to 14 inclusive, was treated in exactly the same way as the controls (Group A), with this exception: from the time of their completion of the quinine and plasmoquine preliminary course until parasites were found in the blood, they received 0.005 gm. of plasmoquine simplex daily. All of them acquired subtertian parasites while still taking this dosage of plasmoquine. All of these subjects were discharged from observation upon the finding of subtertian parasites in the blood. Before the beginning of the quinine-plasmoquine preliminary course of treatment, subjects Nos. 11, 12 and 14 had bloods positive for subtertian malaria; subject No. 13 was negative.

TABLE III. GROUP B

Subjects taking 0.005 gm. plasmoquine daily

Subject no.	Total quinine during preliminary course (in gms.)	No. of days between end of preliminary course and mosquito inoculation	No. of infected mosquitoes used	No. of days between biting by infected mosquitoes and appearance of parasites in blood	RESULT	
11	16.4	36	1	27	Unprotected	0% Protected
12	16.4	34	1	18	Unprotected	
13	16.4	27	1	18	Unprotected	
14	16.4	25	3	19	Unprotected	

GROUP C (Table IV), comprising subjects Nos. 15 to 22 inclusive, were treated in the same manner as the preceding, save that they received a daily dose of 0.01 gm. of plasmoquine simplex beginning immediately after the close of the quinine-plasmoquine preliminary treatment, and continued until their bloods either showed parasites, when they were immediately discharged from observation, or until approximately one month had elapsed after they were bitten by the infected mosquitoes. Subjects Nos. 16, 19, 20, 21 and 22 had subtertian parasites before the preliminary course of treatment; the others were parasite-free. Subject No. 19 is questionable; he will be considered under the discussion of Group D.

TABLE IV. GROUP C

Subjects taking 0.01 gm. plasmoquine daily

Subject no.	Total quinine during preliminary course (in gms.)	No. of days between end of preliminary course and mosquito inoculation	No. of infected mosquitoes used	No. of days between biting by infected mosquito and end of plasmoquine administration	Total no. of days from biting by infected mosquito to discharge from observation	RESULT	
15	11.7	25	4	24	24	Unprotected	12.5% Protected
16	16.8	19	4	31	45	Unprotected	
17	11.7	25	4	41	71	Protected	
18	16.3	31	1	20	20	Unprotected	
19	10.7	25	4	41	66	Unprotected?	
20	16.3	29	1	31	40	Unprotected	
21	16.3	29	1	31	32	Unprotected	
22	16.3	6	1	31	31	Unprotected	

GROUP D (Table V), subjects Nos. 23 to 32 inclusive, were treated similarly to the others, except that their prophylactic dose of plasmoquine was 0.02 gm. a day. Subject No. 23 is the only European included in this experiment. This individual had had 3 previous attacks of subtertian malaria, naturally acquired; the last one about 1½ years previous to this experiment. In this group, subjects Nos. 24, 25, 28 and 31 had subtertian parasites in the blood before the beginning of the quinine-plasmoquine preliminary treatment. The others were negative.

A special precaution against infection by wild mosquitoes was taken in the case of 5 subjects, Nos. 19 and 17 from Group C and Nos. 24, 26 and 32 from Group D, who, 40 days after being bitten by laboratory infected mosquitoes, still had negative bloods. They were then locked in a mosquito-proof house every night before dark and not released until after daylight the following morning. This confinement was continued for a period of 30 days, during which plasmoquine and other medication was stopped.

All remained free of parasites except subject No. 19, who showed both quartan and subtertian parasites on the 25th day of

his confinement, and 66 days after the laboratory inoculation. Since only subtertian sporozoites were used in this experiment, it seems certain that the quartan parasites, at least, came from a wild infection or a relapse from an old infection. Consequently, it is impossible to state positively whether this subject was protected from the laboratory infection or not. However, he is classed as 'unprotected' since he did eventually show parasites.

In order to exclude completely the possibility of wild inoculation during this period, it would have been desirable to place the subjects in the mosquito-proof house several hours before dark; however, since these subjects were all employed, it was impossible to confine them earlier than from about 6.30 p.m. (complete darkness at this season and in this locality coming at about 7 p.m.).

TABLE V. GROUP D

Subjects taking 0.02 gm. plasmoquine daily

Subject no.	Total quinine during preliminary course (in gms.)	No. of days between end of preliminary course and mosquito inoculation	No. of infected mosquitoes used	No. of days between biting by infected mosquito and end of plasmoquine administration	Total no. of days from biting by infected mosquito to discharge from observation	RESULT	
23*	14.6	7	1	11	11	Unprotected	80% Protected
24	11.7	16	4	39	70	Protected	
25	11.7	24	4	14	14	Unprotected	
26	10.7	24	4	42	73	Protected	
27	16.3	14	1	34	75	Protected	
28	16.3	7	2	68	81	Protected	
29	16.3	30	1	31	43	Protected	
30	16.3	31	1	31	42	Protected	
31	16.8	8	1	40	80	Protected	
32	10.1	25	4	41	71	Protected	

* European

GROUP E (Table VI), subjects Nos. 33 to 36 inclusive, were treated in the same way as the preceding groups, except that they received 0.03 gm. of plasmoquine simplex per day. All of them were free of

parasites $1\frac{1}{2}$ months after biting by infected mosquitoes in the laboratory. Subjects Nos. 33, 35 and 36 had subtertian parasites in the blood before the beginning of the quinine-plasmoquine preliminary treatment ; subject No. 34 was negative.

TABLE VI. GROUP E

Subjects taking 0.03 gm. plasmoquine daily

Subject no.	Total quinine during preliminary course (in gms.)	No. of days between end of preliminary course and mosquito inoculation	No. of infected mosquitoes used	No. of days between biting by infected mosquito and end of plasmoquine administration	Total no. of days from biting by infected mosquito to discharge from observations	RESULT	
33	16.3	33	1	30	47	Protected	100% Protected
34	16.3	33	2	30	47	Protected	
35	16.3	35	2	30	45	Protected	
36	16.3	36	1	30	55	Protected	

GROUP F (Table VII), comprising subjects Nos. 37, 38 and 39, were treated in the usual manner except that, in place of plasmoquine simplex, they were given as prophylactic during the mosquito inoculation period 2 tablets of plasmoquine compound per day, a dose equivalent to 0.25 gm. (4 gr.) of quinine and 0.02 gm. of plasmoquine. At the beginning of the experiment, subjects Nos. 37 and 38 had subtertian parasites in the blood ; subject No. 39's blood was negative.

TABLE VII. GROUP F

Subjects taking 2 tablets plasmoquine compound daily

Subject no.	Total quinine during prelim. course (in gms.)	Daily dose plas. com. containing :		No. of days between end of prelim. course and mosquito inoculation	No. of infected mosq. used	No. of days between biting by inf. mosq. and end of plas. com. admin.	Total no. of days from biting by inf. mosq. to dis. from obs.	RESULT	
		quin. (in gms.)	plas. (in gms.)						
37	16.3	0.25	0.02	22	1	31	47	Unprotected	66.6% Protected
38	16.3	0.25	0.02	23	1	31	59	Protected	
39	16.3	0.25	0.02	24	1	31	58	Protected	

GROUP G (Table VIII) consists of 4 subjects, each of whom had been bitten by 4 infected mosquitoes previously and had been protected from infection by plasmoquine (see Tables IV and V, Groups C and D). Each of these 4 subjects was given 0.325 gm. (5 gr.) quinine a day for one month, and then each was re-bitten by 4 infected mosquitoes. The daily dose of 5 gr. of quinine was kept up 10 days after biting. The purpose of this experiment was to get some comparison of the effectiveness of quinine and plasmoquine as a prophylactic in the same individuals. None of these subjects acquired parasites during the 10 days while they were taking quinine, but all of them did show parasites in less than 35 days after quinine had been stopped. Of course, wild infection cannot be ruled out during this 35 days, but it seems unlikely that all of them would have been inoculated by wild infections so quickly

TABLE VIII. GROUP G

Subjects taking 0.325 gm. quinine daily

Subject no.	Daily dose of quinine (in gms.)	No. of infected mosquitoes used	No. of days between biting by infected mosquitoes and end of quinine administration	Total no. of days from biting by infected mosquitoes to appearance of parasites in blood	RESULT	
17	0.325	4	10	44	Unprotected	0 % Protected
24	0.325	4	10	31	Unprotected	
26	0.325	4	10	16	Unprotected	
32	0.325	4	10	43	Unprotected	

GROUP H (Table IX), comprising subjects Nos. 40, 41, 42 and 43, were given the usual 2 weeks' preliminary course of quinine and plasmoquine, following which two of them were given no further medication, and the remaining two were given plasmoquine simplex. These 4 subjects were not bitten by any infected mosquitoes in the laboratory, but in place of this were injected with $1\frac{1}{2}$ c.c. of fresh blood containing large numbers of subtertian schizonts. All injections were intracutaneous. The purpose of this experiment

was to determine whether or not plasmoquine in small daily doses was effective in preventing the development of parasites when mature schizonts were already present, or whether its prophylactic property depends on its action on sporozoites or some pre-schizont stage of the parasites. Subjects Nos. 40 and 41 had negative bloods, and subjects Nos. 42 and 43 had positive bloods before the preliminary course. All of these subjects showed parasites when discharged from observation.

TABLE IX. GROUP II

Subjects injected with blood containing malarial parasites

Subject no.	Age in years	Weight in kilos.	Total quinine during prelim. course (in gms.)	Daily dose of plasmo. during and after inoculation period (in gm.)	Daily dose of plasmo. per kilo. body weight (in mgm.)	No. of days between end of preliminary course and injection of malarial blood	No. of days between injection of malarial blood and end of plasmoquine administration	Total no. of days from injection of malarial blood to disch. from observation	RESULT
40	23	59.5	10.7	0	0	29	0	31	Unprotected
41	21	56.8	11.7	0	0	32	0	8	Unprotected
42	18	54.1	11.7	0.01	0.18	30	32	32	Unprotected
43	16	56.4	11.7	0.02	0.37	30	38	38	Unprotected

Table X gives in summary the data on all the subjects who were bitten by infected mosquitoes while they were taking single daily doses of plasmoquine. The subjects are arranged in order according to the dose of plasmoquine per kilo. of body weight, progressing from the smallest dose per kilo. to the largest.

From the Table, it appears that the minimum effective dose of plasmoquine as a prophylactic is 0.34 mgm. per kilogram of body weight.

THE LENGTH OF TIME A SINGLE DOSE OF PLASMOQUINE REMAINS EFFECTIVE

In our experiments, all subjects took plasmoquine daily before being bitten by infected mosquitoes, so we would have to take into consideration any possible cumulative effect of the drug. In nearly all cases the plasmoquine dose was given at 8 a.m. or soon afterwards, so it is probable that the effect of the dose lasted 24 hours at least

TABLE X

Subjects arranged according to dose of plasmoquine per kilo. body weight

Subject no.	Age in years	Weight in kilos.	Total daily dose plasmo. (in gm.)	Plasmoquine per kilo. body weight per day (in mgm.)	Result
11	18	56.4	0.005	0.09	Unprotected
12	14	50.0	0.005	0.10	Unprotected
13	14	43.2	0.005	0.11	Unprotected
14	14	40.9	0.005	0.12	Unprotected
15	22	61.4	0.01	0.16	Unprotected
16	21	61.4	0.01	0.16	Unprotected
17	28	63.2	0.01	0.16	Protected
18	19	59.1	0.01	0.17	Unprotected
19	25	58.2	0.01	0.17	Unprotected
20	18	55.9	0.01	0.18	Unprotected
21	17	53.6	0.01	0.19	Unprotected
23*	36	77.3	0.02	0.26	Unprotected
37	24	65.5	0.02	0.30	Unprotected
24	25	65.0	0.02	0.31	Protected
22	12	31.8	0.01	0.31	Unprotected
25	22	61.4	0.02	0.33	Unprotected
26	24	60.5	0.02	0.33	Protected
27	20	59.1	0.02	0.34	Protected
28	23	57.7	0.02	0.35	Protected
29	22	57.7	0.02	0.35	Protected
30	23	56.8	0.02	0.35	Protected
38	16	54.5	0.02	0.37	Protected
39	16	54.5	0.02	0.37	Protected
31	21	54.5	0.02	0.37	Protected
32	23	52.7	0.02	0.38	Protected
33	27	70.5	0.03	0.43	Protected
34	15	62.6	0.03	0.48	Protected
36	40	61.4	0.03	0.49	Protected
35	14	50.0	0.03	0.60	Protected

3 out of 17
protected
(inadequate dose)All protected
(effective dose)

* European

in the case of those subjects who were exposed to the bites of wild mosquitoes during the night following the dosage and remained protected.

The time intervening between the dosage of plasmoquine and the exposure to infected mosquitoes in the laboratory is shown in Table XI.

TABLE XI

Time intervening between plasmoquine dosage and exposure to infected mosquitoes in the laboratory

I. UNPROTECTED CASES :

Subjects Nos. 11, 12, 13	Approximately 1 hour
" " 15, 18, 19, 37, 25	" 2 hours
" " 14, 20	" 3 "
" " 16...	" 4 "
" " 21...	" 6½ "
" " 22...	" 7½ "
" " 23...	" 9 "

II. PROTECTED CASES :

Subjects Nos. 24, 26, 32, 36, 34, 33	" 1 hour
" " 17, 30, 39	" 2 hours
" " 35...	" 3 "
" " 31...	" 6 "
" " 27...	" 7 "
" " 28...	" 8 "
" " 38...	" 10 "
" " 29...	" 14 "

The unprotected subjects for the most part received the smaller doses, and the protected, the larger ones (see Table X). Apparently the smaller doses were ineffective even when only 1 or 2 hours intervened between dosage and exposure to infected anopheles, while the effect of the larger doses persisted, in two cases at least as long as 10 hours. The matter is of importance as regards the best time of the day for a prophylactic dose of plasmoquine. It would seem reasonable to take the dose late in the afternoon in order to obtain more protection during the night. These experiments indicate that the size of the dose is of prime importance, as well as the interval between dosage and exposure, that inadequate dosage does not protect even during short intervals, while adequate dosage is effective for many hours.

It may be added that the 13 'protected' individuals who were taking only plasmoquine simplex spent during the period of observation an aggregate of 1,019 nights, or an average of 69 nights each, in unscreened native houses, entirely unprotected from the bites of wild mosquitoes. In a locality such as this, with a very high anopheline intensity and a very high sporozoite rate, it seems most probable that few, if any, of these individuals could have escaped inoculation with malaria by wild mosquitoes.

Since they did escape infection, however, and since the plasmoquine, with 3 or 4 exceptions, was always given in early morning, it would seem that plasmoquine was effective even through the second 12 hours after administration.

ABSENCE OF TOXIC SYMPTOMS DUE TO PLASMOQUINE

During the entire time that plasmoquine was being given, a careful watch was kept for untoward symptoms. There was no case of cyanosis, abdominal discomfort, or any other sign or subjective symptom which could be in any way ascribed to the drug.

In this connection, it may be noted that the writer took 0.02 gm. plasmoquine simplex a day for nearly 3 months; 9 natives took 0.02 gm. a day for an average of 2 months; and 4 other natives took 0.03 gm. per day for 5½ weeks, without the appearance of any symptoms whatever.

INCUBATION PERIOD

The average time elapsing between inoculation and the appearance of parasites in the blood in both controls and those taking inadequate doses of plasmoquine is considerably longer than that generally given for Europeans. This fact is difficult to explain except on the basis of the greater immunity possessed by West Africans.

In the case of the 12 West Africans who took an inadequate dose of plasmoquine simplex and who subsequently developed parasites, the average time elapsing between the time of mosquito inoculation and the appearance of parasites in the blood was 29 days, as compared with 24 days in the case of the 10 controls who took no plasmoquine. In the 'unprotected' subjects, a comparison between those individuals taking the smaller doses (0.16 mgm. or less per kilo. of

body weight daily) and those taking larger doses (0.17 mgm. or more per kilo. of body weight daily) shows that the smaller dosage group developed parasites an average of 25 days after mosquito inoculation, whereas the larger dosage group showed parasites an average of 34 days after mosquito inoculation.

It would seem, then, that within certain limits, even when an inadequate dose of plasmoquine is taken, the appearance of parasites in the blood is delayed ; and that the larger the inadequate dose, the longer the delay in the appearance of parasites.

TABLE XII

Comparison between the groups of West Africans taking inadequate doses of plasmoquine simplex, with reference to the interval elapsing between mosquito inoculation and appearance of parasites in the blood

Group	No. of subjects	Dose of plasmoquine per kilo. body weight	Average no. of days between mosquito inoculation and appearance of parasites in blood
Controls	10	—	24
Smaller inadequate dosage	6	0.09 mgm. to 0.16 mgm.	25
Larger but still inadequate dosage	6	0.17 mgm. to 0.33 mgm.	34
Total inadequate dosage	12	0.09 mgm. to 0.33 mgm.	29

Of all the subjects inoculated, only 5 cases of clinical malaria resulted. These were of the mild type usually seen in people possessing high malarial tolerance.

DISCUSSION

From the beginning we realized that the preliminary course of 1.0 gm. quinine and 0.02 gm. plasmoquine taken over a period of 2 weeks would not necessarily completely eliminate the danger of relapses from old infections in every case. However, in view of the well-known fact that West African natives respond much more quickly and to smaller doses of quinine than do Europeans, it was hoped that parasites would be eliminated at least from a majority of the subjects under experiment.

In any individual case, where a subject showed parasites after experimental inoculation, it cannot be stated positively that these parasites came from the inoculation and not from a relapse due to parasites already present, although absent from the peripheral blood when inoculation occurred. In a large group, however, if relapses were an important factor in this experiment, some of the subjects surely would have relapsed in the time between the end of the preliminary course of treatment and the time of inoculation—an interval in most cases of about a month.

Again, it is untenable that all controls and nearly all individuals taking small doses of plasmoquine should have relapsed, while all those taking comparatively large doses of plasmoquine should have escaped relapse, especially since all subjects were observed for a considerable time after plasmoquine had been stopped.

That plasmoquine in small doses will also protect Europeans, and especially Europeans who have never had malaria, is still unproven. Unfortunately, the only European used in this experiment was given a dose of plasmoquine which subsequently proved to be inadequate for a West African. The experiences of four other Europeans here might be mentioned. None of these people has ever lived in a malarial district before, and none, as far as he knows, has ever had malaria. Two of these people, since their arrival in Liberia 14 months ago, have been taking 1 tablet of quinoplasmine per day at the noon meal. This is equal to a dose of 0.01 gm. plasmoquine and 0.3 gm. quinine per day—a dose of plasmoquine somewhat less than the minimum effective dose for their weight as found in this experiment. However, they have, after 14 months, remained free from malaria. The other two individuals resident here $3\frac{1}{2}$ and $5\frac{1}{2}$ months respectively have taken 2 tablets plasmoquine compound each day at the noon meal—a dose of 0.02 gm. plasmoquine and 0.25 gm. quinine. This dose of plasmoquine is about equal to the minimum effective dose for men of their weight as found in this experiment. Neither of these has had malaria or parasites in the blood. It is worthy of note that one of these men (a radio operator) spends a greater part of every night at work in a place situated less than $\frac{1}{4}$ mile from a native camp containing many native children. Children comprise the greater part of the good gametocyte carriers in this locality. Among other Europeans who take no prophylactic treatment and those taking the

usual 5 gr. quinine per day, practically all contract malaria within the first year, and a majority, especially those who take no prophylaxis, within the first 2 or 3 months of their stay in this locality.

We do not attach any great significance to the attempt at quinine prophylaxis in Group G, because of the possibility that three, at least, of these subjects might have acquired their parasites through wild mosquitoes after the quinine had been stopped. However, the inability of quinine to prevent inoculation of malaria by mosquitoes has been amply demonstrated by other workers.

Likewise, the evidence of Group H, that plasmoquine does not protect when schizonts are directly injected into the subjects, is not entirely conclusive, due to the small number of cases tested.

It must be emphasized that we cannot state positively that the plasmoquine dosage of our 'protected' subjects wholly destroyed the parasites inoculated by the mosquitoes, but the presumption is that it did do so. It is certainly possible that some of the cases might have experienced an attack resulting from the laboratory inoculations after a period of many months, even if entirely free from parasites at the beginning of the experiment and protected against subsequent infections by wild mosquitoes.

It will be noted that in 12 out of 13 of Swellengrebel and de Buck's cases, plasmoquine seemed to give no protection whatever; and the 13th case, which appeared to be protected at first, had an attack accompanied by parasites $7\frac{1}{2}$ months after mosquito inoculation.

We cannot be sure of the explanation for the divergence in the results of Swellengrebel and de Buck's experiment and our own; however, some of the points of difference in the two tests may be pointed out. (1) Swellengrebel and de Buck used Europeans as subjects, most of whom had never had malaria before; we used West Africans, all of whom had had malaria before. (2) Swellengrebel and de Buck used benign tertian parasites, a type of infection notorious for its persistence, while we used malignant tertian. (3) Swellengrebel and de Buck's cases were bitten by from 5 to 12 infected mosquitoes, and therefore had larger doses of sporozoites than our own, which were bitten by from 1 to 4 infected mosquitoes. (4) The plasmoquine administered in Swellengrebel and de Buck's

cases was started 24 hours or less before mosquito inoculation and continued for 5 days, most of their cases having received only 2 doses of 0.01 gm. each of plasmoquine before mosquito inoculation; while our cases, in an effort to imitate as nearly as possible actual field conditions, were given plasmoquine for at least one month before mosquito inoculation and for a month afterwards, and in this way had the advantage of any cumulative effect which the drug might possess.

If, however, in the light of future work it may be demonstrated that plasmoquine can do no more than delay an attack of malaria for several months, it would still be of the greatest service to persons who might thus postpone their primary attacks to winter or until after their work in malarious localities has been finished. It is certain that quinine alone cannot accomplish this result so effectively, at least in the case of subtertian malaria.

CONCLUSION

Tests of the prophylactic effect of plasmoquine were made on a group of West African negroes who had been previously 'sterilized' of malarial parasites by a preliminary treatment with quinine and plasmoquine, and subsequently exposed to the bites of *A. costalis* infected with malignant tertian malaria. The cases may justly be compared to cured cases of malaria, and it seems likely that the conclusion would also hold for persons who have never had malaria. However, there is no direct evidence in this experiment on this latter point.

The results indicate:

1. That a daily dosage of as much as 3 centigrams of plasmoquine over a period of several months is not followed by any untoward symptoms in those taking it.
2. That a daily dosage of 0.33 mgm. or less per kilo. of body weight does not give adequate protection, while a daily dosage of 0.34 mgm. or more per kilo. of body weight protects those individuals taking it. On this basis, the minimum effective dose of plasmoquine

for a man of 150 lbs. weight would be 2.3 centigrams per day, best taken after the noon meal.

3. That plasmoquine may prove to be an effective malarial prophylactic for people living in districts highly infected with subtertian malaria seems a distinct possibility.

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STUDIES IN CHEMOTHERAPY*
VIII. COMPARISON OF STRAINS OF
T. RHODESIENSE MADE RESISTANT TO
VARIOUS ARSENICALS AND ANTIMONIALS,
TO BAYER 205, AND TO ACRIFLAVINE,
RESPECTIVELY

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In previous papers (1930 and 1931), we have shown that a strain of *T. rhodesiense* made resistant to atoxyl is indistinguishable from a strain of the same parasite made resistant to acriflavine. The response of each of these two strains to various compounds of arsenic and antimony, to acriflavine, and to Bayer 205, although often very different from that of the normal parent strain, proved in every instance to be exactly similar to that of the other. Broadly speaking, the two resistant strains exhibited marked resistance to all the aromatic compounds of arsenic and antimony, and to acriflavine, but were just as sensitive to sodium arsenite, tartar emetic, and Bayer 205 as was the normal strain. But the similarity of the two resistant strains was even closer than this, in that they each exhibited the same differences in degree of resistance to the various aromatic compounds of arsenic and antimony: e.g., to arsenophenylglycine they were less than twice as resistant as was the normal strain; to reduced atoxyl thioglycollate 4 to 8 times as resistant; to reduced arsacetin, halarsol and novarsenobillon about 32 times as resistant; and to reduced tryparsamide about 256 times as resistant.

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These observations have led us to inquire, firstly, whether it is also possible, by treatment of infected animals with subcurative doses of the other commonly employed aromatic arsenical and antimonial compounds, of tartar emetic, and of Bayer 205, to produce resistant strains; and secondly, whether the resulting strains are similar to the atoxyl-fast and acriflavine-fast strains. The compounds* used in these experiments were:—

- A. *Aromatic compounds of arsenic*.—Atoxyl, arsacetin, tryparsamide, reduced tryparsamide thioglycollate, halarsol, novarsenobillon and arsenophenylglycine.
- B. *Aromatic compound of antimony*.—Stibenyl.
- C. *Non-aromatic compound of antimony*.—Tartar emetic.
- D. Acriflavine.
- E. Bayer 205.

In an endeavour to produce strains of *T. rhodesiense* resistant to each of these compounds, we adopted the general procedure used for the preparation of the atoxyl- and acriflavine-fast strains. Infected mice were treated with subcurative doses of the drugs, and the doses were gradually increased as relapses occurred in these animals and in animals of later passage.

Eventually we succeeded in producing resistant strains with every drug except tartar emetic. So far as could be judged from *in vivo* experiments, the degree of resistance ultimately reached was complete in all the strains except the arsenophenylglycine- and stibenyl-fast strains; i.e., in every case, except these two, the resistant strain withstood the maximum tolerated dose of the drug used to produce it. The atoxyl-fast strain withstood 10 mgm. of atoxyl, the arsacetin-fast strain 40 mgm. of arsacetin, the tryparsamide-fast strain 60 mgm. of tryparsamide, the reduced tryparsamide-fast strain 2.5 mgm. of reduced tryparsamide, the halarsol-fast strain 0.75 mgm. of halarsol, the novarsenobillon-fast strain 4 mgm. of novarsenobillon, the acriflavine-fast strain 0.4 mgm. of acriflavine, and the Bayer 205-fast strain 10.0 mgm. of Bayer 205.† Each of these doses is the maximum dose tolerated by a 20 gm. mouse.

* The structural formulae and correct chemical names are given in a previous paper (1931).

† Notwithstanding the fact that the M.E.D. of Bayer 205 for the Bayer 205-fast strain was about 400 times that for the normal strain, the minimum trypanocidal concentration *in vitro* of this drug was but slightly greater for the resistant strain than for the normal strain. This fact, together with its relatively slight trypanocidal action *in vitro*, suggests that Bayer 205 exerts its therapeutic activity only after it has been changed in the body of the host.

In the case of the arsenophenylglycine-fast strain, the resistance reached, although considerable, was not complete; the strain withstood 1.0 mgm. of arsenophenylglycine (the M.E.D. for the normal strain is 0.5 mgm.), but not 1.5 mgm., which is the maximum tolerated dose for a 20 gm. mouse. Similarly, the stibenyl resistant strain withstood 10 mgm. of stibenyl (the M.E.D. for the normal strain being 4 mgm.), but not 15 mgm., which approaches the maximum tolerated dose for a 20 gm. mouse.

It is interesting to note that the time required for the production of these different resistant strains varied considerably (Table I).

TABLE I

Showing the length of time required for the production of resistant strains by the use of various compounds

Compound	Date of first dose of compound	Date of last dose of compound*	Date when a definite degree of resistance was first observed†	Date when maximum degree of resistance was observed
Atoxyl	12.2.29	19.8.30	7.3.29	21.3.29
Arsacetin	5.2.31	17.6.31	25.3.31	9.4.31
Tryparsamide	5.2.31	24.3.31	4.3.31	10.3.31
Red tryparsamide thioglycollate	5.2.31	10.4.31	3.3.31	11.3.31
Halarsol	17.4.31	12.9.31	28.7.31	28.8.31
Novarsenobillon	21.4.31	22.8.31	20.7.31	1.8.31
Arsenophenylglycine	26.2.31	16.2.32	15.12.31	8.1.31
Stibenyl	30.4.31	17.9.31	11.6.31	2.7.32
Tartar emetic, 1st strain ...	5.2.31	20.10.31	No resistance	developed
„ „ 2nd strain ...	3.11.31	20.4.32	No resistance	developed
Acriflavine	4.7.28	18.8.30	18.7.28	15.8.28
Bayer 205... ..	2.5.31	30.9.32	7.12.31	30.9.32
Atoxyl-emetic	17.3.32	7.4.32	21.3.32	23.3.32

* It will be observed that in many instances we continued to give the drug for prolonged periods after the strain had attained to its maximum degree of resistance. The object of this was to make certain that the maximum degree of resistance had actually been reached.

† By a definite degree of resistance we mean a stage when about twice the minimum effective dose for the normal strain failed to exert any influence on the infection.

The atoxyl-fast, arsacetin-fast, tryparsamide-fast, reduced tryparsamide-fast, stibenyl-fast and acriflavine-fast strains were all obtained within a period of 4-8 weeks; the halarsol-fast and novarsenobillon-fast strains required about 3 months for their production; the arsenophenylglycine-fast strain developed still more slowly; and the Bayer 205-fast strain did not attain to its maximum degree of resistance until after more than 12 months. It is possible, however, that to some extent these differences may be more apparent than real, because we have obtained evidence, with which we shall deal in a later paper, that in the case of any one drug, e.g. tryparsamide, the rapidity with which resistant strains are produced varies greatly according to the particular technique adopted.

Having satisfied ourselves that these various strains had attained to a degree of resistance which could not be increased, we decided to compare them by examining the reactions of each strain *in vitro* to reduced tryparsamide, halarsol, arsenophenylglycine and emetic, and *in vivo* to these drugs and also to stibenyl and Bayer 205.

The results of this work are summarized in Tables II and III, from which the following points emerge:—

1. The strain which we had unsuccessfully endeavoured to make resistant to tartar emetic was identical in all respects with the normal parent strain.
2. The atoxyl-fast, arsacetin-fast, tryparsamide-fast, reduced tryparsamide-fast, halarsol-fast, novarsenobillon-fast, and acriflavine-fast strains were indistinguishable from one another, in that they all proved, both by *in vivo* and *in vitro* tests, to be highly resistant to reduced tryparsamide, halarsol and stibenyl, to be but slightly resistant to arsenophenylglycine, and to be just as sensitive to tartar emetic and Bayer 205 as was the normal parent strain.
3. The stibenyl-fast strain was similar to the above resistant strains, except that it was also resistant to tartar emetic.
4. The arsenophenylglycine-fast strain exhibited only a moderate degree of resistance to reduced tryparsamide, halarsol and stibenyl; it proved considerably more resistant to arsenophenylglycine than any of the other strains; and it was just as sensitive to tartar emetic and Bayer 205 as was the normal strain.
5. The Bayer 205-fast strain exhibited almost complete resistance to Bayer 205, but was normally sensitive to all the other drugs.

TABLE II

Showing the concentration of various drugs necessary to destroy *in vitro* within 6 hours at 37° C. trypanosomes of the normal and of the different resistant strains, respectively

Drug used in attempt to produce resistant strain	Reduced tryparsamide	Halarsol	Arsenophenylglycine	Tartar emetic
(Normal strain) ...	1 : 12,800,000/25,600,000	1 : 12,800,000/25,600,000	1 : 800,000	1 : 3,200,000
Atoxyl ...	1 : 50,000/100,000	1 : 400,000/800,000	1 : 400,000	1 : 3,200,000
Arsacetin ...	1 : 200,000	1 : 400,000/800,000	1 : 400,000	1 : 3,200,000
Tryparsamide...	1 : 50,000/100,000	1 : 400,000/800,000	1 : 400,000	1 : 3,200,000
Reduced tryparsamide	1 : 50,000/100,000	1 : 400,000/800,000	1 : 400,000	1 : 3,200,000
Halarsol ...	1 : 50,000/100,000	1 : 400,000/800,000	1 : 400,000	1 : 3,200,000
Novarsenobillon ...	1 : 50,000/100,000	1 : 400,000/800,000	1 : 400,000	1 : 3,200,000
Arsenophenylglycine	1 : 3,200,000/6,400,000	1 : 3,200,000/6,400,000	1 : 400,000	1 : 3,200,000
Stibenyl ...	1 : 50,000/100,000	1 : 400,000	1 : 400,000	1 : 800,000/1,600,000
Emetic...	1 : 12,800,000/25,600,000	1 : 12,800,000/25,600,000	1 : 800,000	1 : 3,200,000
Acriflavine ...	1 : 50,000/100,000	1 : 400,000/800,000	1 : 400,000	1 : 3,200,000
Bayer 205 ...	1 : 12,800,000/25,600,000	1 : 12,800,000/25,600,000	1 : 800,000	1 : 3,200,000
Atoxyl-emetic ...	1 : 50,000/100,000	1 : 400,000/800,000	1 : 400,000	1 : 1,600,000

TABLE III

Showing the minimum effective dose of various drugs for 20 gm. mice infected with the normal, and with the different resistant strains, respectively

Drug used in attempt to produce resistant strain	Reduced tryparsamide mgm.	Halarsol mgm.	Arsenophenylglycine mgm.	Acriflavine mgm.	Tartar emetic mgm.	Stibenyl mgm.	Bayer 205 mgm.
(Normal strain) ...	0.04	0.025	0.5	0.1	0.4	4.0	0.025
Atoxyl ...	2.5++	0.75++	0.6	0.4++	0.4	15.0	0.025
Arsacetin ...	2.5++	0.75++	0.6	—	0.4	15.0	0.025
Tryparsamide...	2.5++	0.75++	0.6	0.4++	0.4	15.0	0.025
Reduced tryparsamide	2.5++	0.75++	0.6	—	0.4	15.0	0.025
Halarsol ...	2.5++	0.75++	0.6	—	0.4	15.0	0.025
Novarsenobillon ..	2.5++	0.75++	0.6	—	0.4	15.0	0.025
Arsenophenylglycine	0.75	0.2	1.5	0.4+	0.4	10.0	0.025
Stibenyl ...	2.5++	0.75++	0.6	—	1.0++	15.0	0.025
Emetic...	0.04	0.025	0.5	—	0.4	4.0	0.025
Acriflavine ...	2.5++	0.75++	0.6	0.4++	0.4	15.0	0.025
Bayer 205 ...	0.04	0.025	0.5	0.1	0.4	4.0	15.0
Atoxyl-emetic ...	2.5++	0.75++	0.6	—	1.0++	15.0	0.025

These facts enable us to divide the resistant strains into four groups :—

(a) Strongly resistant to the aromatic compounds of arsenic (except arsenophenylglycine) and of antimony ; slightly resistant to arsenophenylglycine ; sensitive to tartar emetic and to Bayer 205, viz. :—*atoxyl-fast*, *arsacetin-fast*, *tryparsamide-fast*, *reduced tryparsamide-fast*, *halarsol-fast*, *novarsenobillon-fast*, and *acriflavine-fast* strains.

(b) Resistant to arsenophenylglycine ; moderately resistant to the other aromatic arsenicals and antimonials ; sensitive to tartar emetic and to Bayer 205, viz.—*arsenophenylglycine-fast* strain.

(c) Similar to Group A, but resistant to tartar emetic, viz. *stibenyl-fast* strain.

(d) Strongly resistant to Bayer 205 ; sensitive to all the other drugs, viz. *Bayer 205-fast* strain.

Apparently, therefore, resistance to the aromatic arsenical or antimonial compounds does not imply resistance to Bayer 205, and, conversely, resistance to the latter does not mean resistance to the former. Resistance to one aromatic arsenical does, however, involve resistance to other aromatic arsenicals, and also to the aromatic antimonials and to acriflavine. But although this generalization is true, it does not follow that a strain made resistant to one aromatic compound of arsenic is necessarily identical with that made resistant to another. Whilst it seems impossible to distinguish between strains made resistant to atoxyl, arsacetin, tryparsamide, halarsol, novarsenobillon and acriflavine respectively, yet these strains are clearly distinguishable from a strain made resistant to arsenophenylglycine, because the latter is much more resistant to arsenophenylglycine, and much less resistant to the other aromatic arsenicals and antimonials, than are the former.

Again, it is interesting to note that there is a very important difference between a strain resistant to an aromatic arsenical, such as arsacetin, and one resistant to its antimony analogue stibenyl. The former is sensitive to emetic, and the latter is resistant. This fact at first sight conflicts with the general conclusion reached in one of our previous papers (1930), viz. :—that the strains are resistant to the substituted phenyl radical of the aromatic

compounds, and not to arsenic or antimony. An arsacetin-fast strain is not resistant to sodium arsenite, and consequently at first sight it is not easy to understand why a stibenyl-fast strain should prove resistant to the non-aromatic antimonial tartar emetic; and this is the more perplexing, as endeavours to produce a resistant strain by prolonged administration of tartar emetic have proved futile—as also have those of many previous workers (Mesnil and Brimont, 1908, Ehrlich, 1908, Heckenroth, 1910, and Morgenroth and Rosenthal, 1911).

Notwithstanding the extreme difficulty, or perhaps impossibility, of producing a tartar emetic-fast strain by the direct method of administering this drug to animals infected with the normal strain, it has been shown by Mesnil and Brimont (1908), Ehrlich (1908) and others, that a tartar emetic-fast strain can readily be made by the indirect method of first making a strain resistant to atoxyl, and then treating mice infected with the atoxyl-fast strain in the usual manner with subcurative doses of tartar emetic. We repeated this work, using our old atoxyl-fast strain, and found that a strain, which resisted the maximum dose (1.0 mgm.) of tartar emetic tolerated by a 20 gm. mouse, could be produced with remarkable speed; two or three doses of tartar emetic sufficed to produce a strain completely resistant to the maximum dose (1.0 mgm.) tolerated by a 20 gm. mouse.

This observation, which has possibly not received the attention which it merits, may have an important bearing on the therapy of man. In any case, one may with justice inquire whether those who so ardently advocate the advantages, in the treatment of human trypanosomiasis, of a mixed treatment consisting of atoxyl and emetic, have been aware of the fact that when once a strain has become atoxyl-fast it quickly becomes emetic-fast. However, these considerations are outside the scope of the present paper; we merely wish to point out that such a strain, which in the tables we designate as the atoxyl emetic-fast strain, is identical in all respects with the stibenyl-fast strain, in that it is resistant not only to the aromatic compounds of arsenic and antimony but also to tartar emetic. We believe that this fact throws light on the mechanism whereby a stibenyl-fast strain is also emetic-fast. The organic compounds of antimony are notoriously unstable, and it

seems not unreasonable to assume that stibenyl, after its incorporation into the body of the host, decomposes to some extent, with the result that a certain quantity of a non-aromatic trivalent antimonial is set free. Such a hypothesis would afford, on the analogy of the atoxyl emetic-fast strain, a simple explanation of the fact that a stibenyl-fast strain is also emetic-fast.

SUMMARY

1. The object of the work described in this paper was, firstly, the preparation of a number of strains resistant to various drugs, and secondly, the comparison of the resulting resistant strains to see how far they differed from one another and from the normal parent strain.

The normal strain used was an old laboratory strain of *T. rhodesiense*, and the technique employed in the endeavour to produce the resistant strains was the customary one of treating mice infected with the normal strain with subcurative doses of the drugs, and gradually increasing the doses as relapses occurred in the original mice and in those of later passage. The compounds to which it was attempted to produce resistant strains were:—

- A. *Aromatic compounds of arsenic*.—Atoxyl, arsacetin, tryparsamide, reduced tryparsamide thioglycollate, halarsol, novarsenobillon and arsenophenylglycine.
- B. *Aromatic compound of antimony*.—Stibenyl.
- C. *Non-aromatic compound of antimony*.—Tartar emetic.
- D. *Acridine*.
- E. *Bayer 205*.

2. By this procedure it was found possible to produce resistant strains with every drug, except tartar emetic.

3. The speed with which resistant strains were produced varied considerably. With atoxyl, arsacetin, tryparsamide, reduced tryparsamide thioglycollate, stibenyl and acridine, highly resistant strains were produced within 4 to 8 weeks. The production of halarsol- and novarsenobillon-fast strains required a period of about 3 months; the arsenophenylglycine-fast strain developed still more slowly; and the Bayer 205-fast strain did not attain to its maximum degree of resistance until after more than 12 months.

For reasons stated, however, it is possible that to some extent these differences may be more apparent than real.

4. Comparison of the resulting strains showed that they could be arranged into the following 4 groups :—

(a) Strongly resistant to the aromatic compounds of arsenic (except arsenophenylglycine) and of antimony ; slightly resistant to arsenophenylglycine ; sensitive to tartar emetic and to Bayer 205, viz.—*atoxyl-fast*, *arsacetin-fast*, *tryparsamide-fast*, *reduced tryparsamide-fast*, *halarsol-fast*, *novarsenobillon-fast*, and *acriflavine-fast* strains.

(b) Resistant to arsenophenylglycine ; moderately resistant to the other aromatic arsenicals and antimonials ; sensitive to tartar emetic and to Bayer 205, viz.—*arsenophenylglycine-fast* strain.

(c) Similar to Group A, but resistant to tartar emetic, viz.—*stibenyl-fast* strain.

(d) Strongly resistant to Bayer 205 ; sensitive to all the other drugs, viz.—*Bayer 205-fast* strain.

5. These facts appear to admit of the following generalizations :

(a) Resistance to Bayer 205 does not involve resistance to arsenical or antimonial compounds, and conversely resistance to the latter does not mean resistance to the former.

(b) Resistance to an aromatic arsenical or antimonial compound implies resistance to other aromatic arsenicals and antimonials ; but this does not necessarily mean that a strain made resistant to one aromatic arsenical is identical with that made resistant to another, *vide* the differences in degrees of resistance exhibited by a tryparsamide-fast strain and a strain made resistant to arsenophenylglycine.

(c) A strain made resistant to an aromatic arsenical is sensitive to tartar emetic, whereas a strain made resistant to an aromatic antimonial is resistant to tartar emetic. This fact calls for special comment. Although it is extremely difficult, or even impossible, to produce a tartar emetic-resistant strain by the ordinary direct method of administering subcurative doses of the drug to mice infected with the normal strain, it is exceedingly easy to produce a resistant strain by the indirect method of first producing an atoxyl-resistant strain and then acting upon it with tartar emetic.

There is evidence to believe that stibenyl, like the other aromatic antimonial compounds, readily undergoes a certain amount of decomposition when introduced into the animal body; and we believe that the mechanism, whereby a stibenyl-fast strain becomes emetic-fast, is similar to that whereby an atoxyl-fast strain can subsequently be made emetic-fast.

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STUDIES IN CHEMOTHERAPY*

IX. SODIUM THIOSULPHATE AND ARSENIC-RESISTANCE

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In a recent paper, Citron (1930) claims that he has been able to destroy the resistance of a salvarsan-fast strain of trypanosomes by prolonged treatment of a series of infected mice with sodium thiosulphate. His exact technique was as follows :—

The strain of *T. brucei* used had attained to a high degree of resistance to neosalvarsan because, although infected mice were usually sterilized by a dose of 8 mgm. per 20 gm., smaller doses of 4 mgm. had no effect.

Four mice were inoculated with the strain, and on the next day, when the blood contained about 1 trypanosome per 5 microscope fields, one of them was given an intravenous injection of 1 c.c. of a 2.5 per cent. solution of sodium thiosulphate per 20 gm. mouse; this was about the maximum tolerated dose and it had no influence on the course of the infection. The remaining three mice were given respectively 8 mgm., 4 mgm. and 2 mgm. of neosalvarsan per 20 gm. mouse.

When the mouse which had received the sodium thiosulphate showed 3-10 parasites to a field, its blood was inoculated into another group of 4 mice. One of these was given sodium thiosulphate, and the other three tested with neosalvarsan as described above.

The process was continued, and no change in the resistance of the strain was noticed during the first 17 passages. At the 18th passage, however, there appeared a sudden increase in the sensitiveness of the strain to neosalvarsan. Whilst previously the infection was uninfluenced by a dose of 4 mgm. of neosalvarsan per 20 gm. mouse, it was now observed that a dose of only 0.125 mgm. per 20 gm. sufficed to cure. As this was the minimum curative dose for the normal strain it would appear that under the influence of sodium thiosulphate the resistance of the salvarsan-fast strain had been completely lost.

In a second experiment in all respects similar to that just described, exactly parallel results were obtained. The resistance was fully maintained during the first seventeen passages, but from the eighteenth passage onwards the resistance was completely lost.

Kritschewski and Demidowa (1931) used Citron's technique in an attempt to make three strains of spirochaetes more sensitive

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to the action of salvarsan. They found that after 17 exposures to sodium thiosulphate the spirochaetes were so modified that infected mice would invariably be sterilized by a dose of 2.5 mgm. of salvarsan per 20 gm., whereas mice infected with the original strains were very rarely sterilized by this dose.

The experiments described in the present paper were performed with the object of confirming Citron's interesting observation. In our first experiment we employed our old atoxyl-fast strain, the genesis of which is fully given in a previous paper (Yorke and Hawking, 1932).

EXPERIMENT. On 12th September, 1931, two mice were inoculated subcutaneously with the atoxyl-fast strain from Passage 283, i.e. 129 passages after the last exposure of the strain to atoxyl on 19th August, 1930. On the day of inoculation and on each of the following two days 0.25 c.c. per 20 gm. mouse of a 10 per cent. solution of sodium thiosulphate (25 mgm.) was injected intraperitoneally (Table I, Exp. 1). On the next day two mice were inoculated subcutaneously from Mouse A, and two from Mouse B. Of the two inoculated from Mouse A, one was given 25 mgm. of sodium thiosulphate on the first and second days after inoculation, and the other was given a maximum tolerated dose of reduced tryparsamide thioglycollate on the second day in order to test the resistance of the strain. Similarly, in the case of the mice infected from Mouse B, one was given sodium thiosulphate and the other tested with reduced tryparsamide thioglycollate (Table I, Exp. 2). On the third day two mice were infected from each of the mice which had been given sodium thiosulphate, and one of them was given sodium thiosulphate and the other one used for testing the resistance of the strain.

In this manner the experiment was continued until each strain (A and B) had been exposed to sodium thiosulphate in a series of 50 mice.* Four mice were then infected with each strain and the infections tested against maximum tolerated doses of reduced tryparsamide, halarsol, atoxyl and N.A.B., with the results shown in Table I, Exp. 51.

Concurrently with these experiments two other series of experiments were performed. These were exactly similar to those already described, except that we used a strain of *T. rhodesiense* made resistant to novarsenobillon. The development of this strain from the normal strain of *T. rhodesiense* is shown in Table II. Details of the administration of sodium thiosulphate in the first 3 mouse passages, and the result of testing the strain after it had been subjected to the influence of the sodium thiosulphate in 50 mouse passages, are shown in Table III.

Reference to Table I, Exp. 51, and Table III, Exp. 51, shows that, contrary to the experience of Citron, the repeated

* In most of these mice three doses of sodium thiosulphate, but in a few cases only two doses, were given.

TABLE I

Showing the effect of prolonged treatment with sodium thiosulphate on the resistance of an atoxyl-fast strain of *T. rhodesiense*

Exp. 1. Showing administration of sodium thiosulphate to mice of 1st passage.

Mouse	Inoculated from	Result of examination of blood of mice, and effect of treatment				
		Day after inoculation				
		0	1	2	3	4
A	Atoxyl-fast strain Passage 283	Thio. 25	1/2 Thio. 25	10 Thio. 25	+ Exp. 2	+++ Dead
B		Thio. 25	1/3 Thio. 25	+ Thio. 25	+ Exp. 2	+++ Dead

Exp. 2. Showing the administration of sodium thiosulphate to mice of 2nd passage; and tests of the resistance of strains to reduced trypanamide.

Mouse	Inoculated from	Result of examination of blood of mice, and effect of treatment				
		Day after inoculation				
		1	2	3	4	
A 1	Exp. 1 Mouse A	1/40 Thio. 25	1 Thio. 25	++ Exp. 3		Dead
A 2		1/50	5 R.T. 2.5	++		Dead
B 1	Exp. 1 Mouse B	— Thio. 25	+ Thio. 25	++ Exp. 3		Dead
B 2		—	—	10 R.T. 2.5		Dead

Exp. 3. Showing the administration of sodium thiosulphate to mice of 3rd passage; and tests of resistance of strains to reduced trypanamide.

Mouse	Inoculated from	Result of examination of blood of mice, and effect of treatment					
		Day after inoculation					
		0	1	2	3	4	5
A 1	Exp. 2 Mouse A.1	Thio. 25		— Thio. 25	1/2 Thio. 25	+ Exp. 4	++ Dead
A 2				—	1/20 R.T. 2.5	10	+ Dead
B 1	Exp. 2 Mouse B.1	Thio. 25		3 Thio. 25	+ Thio. 25	++ Exp. 4	Dead
B 2				1 R.T. 2.5	8 Dead		

TABLE I—*continued*

Exp. 51. Showing the resistance to various drugs after the atoxyl-fast strains had been exposed to sodium thiosulphate during 50 passages in mice.

Mouse	Inoculated from	Result of examination of blood of mice, and effect of treatment					
		Day after inoculation					
		1	2	3	4	5	6
A 1	Exp. 50 Mouse A.1		2	20 R.T. 2.5	++	Dead	Dead
A 2			1/10	1 Hal. 0.75	10	+	
A 3			2 Atoxyl 10	++	++	Dead	
A 4			10 N.A.B. 4	+ Dead			
B 1	Exp. 50 Mouse B.1		1/2	10 R.T. 2.5	++	Dead	++ Dead
B 2			1	5 Hal. 0.75	5	+	
B 3		1/10	+ Atoxyl 10	++	Dead		
B 4			15 N.A.B. 4	+	5	Dead	

N.B. 1/2, 10, +, etc. = number of trypanosomes per microscope field.

— = blood negative.

Thio. 25 = sodium thiosulphate 25 mgm. per 20 gm. mouse.

R.T. 2.5 = reduced trypanamide thioglycolate 2.5 mgm. per 20 gm. mouse.

Hal. 0.75 = halarsol 0.75 mgm. per 20 gm. mouse.

Atoxyl 10 = atoxyl 10.0 mgm. per 20 gm. mouse.

N.A.B. 4 = novarsenobillon 4.0 mgm. per 20 gm. mouse.

administrations of sodium thiosulphate during 50 mouse passages failed to produce any recognizable reduction in the degree of resistance of either the atoxyl- or the novarsenobillon-fast strain. It is not easy to understand the reason for this pronounced divergence of results. Citron found, in each of his two experiments, that the resistance of his neosalvarsan-fast strain disappeared after the strain had passed through 17 mice treated with sodium thiosulphate, whereas in none of our four experiments—two with an atoxyl-fast strain and two with a novarsenobillon-fast strain—was any decrease in the resistance noticed even after 50 mouse passages. The only obvious differences between Citron's experiments and ours were that he used a neosalvarsan-fast strain and administered to each infected mouse a single dose (25 mgm. per 20 gm.) of sodium thiosulphate intravenously, whereas we used atoxyl- and novarsenobillon-fast strains and gave several doses (25 mgm. per 20 gm.) of sodium thiosulphate intraperitoneally to each mouse. It is probable

TABLE II

Showing the development of the N.A.B.-resistant strain of *T. rhodesiense* in mice.

Mouse	Date of treatment	Dose of N.A.B. per 20 gm. mouse	Result of treatment
1	21.4.31	0.25	Negative 8 days
	30.4.31	0.375	" 17 "
	19.5.31	0.375	" 14 "
	5.6.31	0.375	" 5 "
2	No treatment		
3	18.6.31	0.2	Negative 2 days
4	25.6.31	0.2	" 7 "
	7.7.31	0.2	" 3 "
	13.7.31	0.2	" 6 "
	21.7.31	0.2	Not negative
	22.7.31	0.2	" "
5	27.7.31	0.4	No action
6	31.7.31	2.5	" "
7	6.8.31	2.5	" "
	7.8.31	2.5	Negative 2 days
8	No treatment		
9	17.8.31	3.0	No action
10-16	No treatment		
17			14-9-31. Off-shoot for thiosulphate experiments

TABLE III

Showing the effect of prolonged treatment with sodium thiosulphate on the resistance of a novarsenobillon-fast strain of *T. rhodesiense*.

Exp. 1. Showing administration of sodium thiosulphate to mice of 1st passage.

Mouse	Inoculated from	Result of examination of blood of mice, and effect of treatment			
		Day after inoculation			
		1	2	3	4
A	N.A.B.-fast strain Passage 17	- Thio. 25	3 Thio. 25	++ Exp. 2	Dead
B		- Thio. 25	1/10 Thio. 25	++ Exp. 2	Dead

TABLE III—continued

Exp. 2. Showing the administration of sodium thiosulphate to mice of 2nd passage; and tests of resistance of strain to reduced tryparsamide.

Mouse	Inoculated from	Result of examination of blood of mice, and effect of treatment					
		Day after inoculation					
		1		2		4	
A 1	Exp. 1 Mouse A	—	Thio. 25	1/15	Thio. 25	++	Exp. 3
A 2		—		—		++	R.T. 2.5
B 1	Exp. 1 Mouse B	—	Thio. 25	—	Thio. 25	++	Exp. 3
B 2		—		1/20	R.T. 2.5	++	Dead

Exp. 3. Showing the administration of sodium thiosulphate to mice of 3rd passage; and tests of resistance of strains to reduced tryparsamide.

Mouse	Inoculated from	Result of examination of blood of mice, and effect of treatment					
		Day after inoculation					
		0	1	2	3	4	
A 1	Exp. 2 Mouse A.1	—Thio. 25	—	Thio. 25	1/5	Thio. 25	+ Exp. 4
A 2		—	—		5	R.T. 2.5	++
B 1	Exp. 2 Mouse B.1	—Thio. 25	1/30	Thio. 25	1/5	Thio. 25	++ Exp. 4
B 2		—	—		3	R.T. 2.5	+

Exp. 51. Showing the resistance to various drugs after the novarsenobillon-fast strains had been exposed to sodium thiosulphate during 50 passages in mice.

Mouse	Inoculated from	Result of examination of blood of mice, and effect of treatment					
		Day after inoculation					
		2	3	4	5	6	
A 1	Exp. 50 Mouse A.1	1/20	10	R.T. 2.5	+	Dead	
A 2		1/3	2	Hal. 0.75	++	++	Dead
A 3		5 Atoxyl 10	+		++	Dead	
A 4		—	20	N.A.B. 4.0	+	1/2	Dead
B 1	Exp. 50 Mouse B.1	—	1/2	R.T. 2.5	10	+	Dead
B 2		—	1/5	Hal. 0.75	10	++	++ Dead
B 3		1/5 Atoxyl 10	2		+	Dead	
B 4		—	1	N.A.B. 4.0	20	20	++ Dead

that a single intravenous injection of 25 mgm. of sodium thiosulphate produced, momentarily, a higher concentration of the drug in the blood of the mouse than did intraperitoneal injections of the same dose given on each of 2 or 3 consecutive days; but by the latter procedure the trypanosomes would certainly be exposed to the action of the drug for a longer period.

With a view to ascertaining whether the highest possible concentration of sodium thiosulphate would be more effective, we performed experiments in which an atoxyl-resistant strain of trypanosomes was repeatedly exposed to 5 to 10 per cent. solutions *in vitro*—each exposure *in vitro* alternating with the passage of the parasites through a normal mouse.

EXPERIMENT. A heavy suspension, in nutrient medium (Yorke *et al.*, 1929), of our atoxyl-resistant strain of *T. rhodesiense* was prepared from an infected mouse. An equal volume (0.5 c.c.) of this suspension was then added to each of four tubes containing respectively nutrient medium alone, and nutrient medium in which was dissolved 1:5, 1:10, and 1:25 of sodium thiosulphate. After incubation at 37° C. for one hour, the contents were examined (Table IV, Exp. 1a) and injected intraperitoneally into mice (Table IV, Exp. 1b). One of these mice was used to supply the trypanosomes for the next *in vitro* exposure to sodium thiosulphate, and the remainder were used for the purpose of ascertaining the resistance of the strain to reduced tryparsamide. This procedure was repeated 22 times, after which the experiment was abandoned (Table IV, Exp. 22b).

TABLE IV

Showing the effect on the resistance of an atoxyl-fast strain of *T. rhodesiense* of repeated exposures *in vitro* to sodium thiosulphate.

Exp. 1a. Showing the result of Exposure 1.

Tube	Concentration of sodium thiosulphate	Source of trypanosomes	Number of trypanosomes per 256 squares of the haemocytometer scale at beginning of experiment	Condition of trypanosomes after 1 hour's exposure at 37° C.
1	1 : 10	Atoxyl-fast strain Passage 337	5,000	All motionless
2	1 : 20			About 100 motile
3	1 : 50			All motile
4	Control			All motile

TABLE IV—continued

Exp. 1*b*. Showing the result of injection of mice with trypanosomes after Exposure 1, and the resistance of the infection to reduced tryparsamide.

Mouse	Inoculum	Result of examination of blood of mice, and effect of treatment					
		Day after inoculation					
		2	3	4	5	6	7
116 A	Exp. 1 <i>a</i>	—	—	1/30	20	+ Exp. 2	
B	Tube 1	—	—	—	20 R.T. 2.5	++	Dead
116 C	Exp. 1 <i>a</i>	—	1/5	+ R.T. 2.5	+++	Dead	
D	Tube 2	—	8	++ R.T. 2.5	+++	Dead	

Exp. 2*a*. Showing the result of Exposure 2.

Tube	Concentration of sodium thiosulphate	Source of trypanosomes	Number of trypanosomes per 256 squares of the haemocytometer scale at beginning of experiment	Condition of trypanosomes after 1 hour's exposure at 37° C.
1	1 : 10	Mouse 116A	500	All motionless
2	1 : 20	Exp. 1 <i>b</i> ...		About 200 motile

Exp. 2*b*. Showing the result of injection of mice with trypanosomes after Exposure 2, and the resistance of the infection to reduced tryparsamide.

Mouse	Inoculum	Result of examination of blood of mice, and effect of treatment					
		Day after inoculation					
		4	5	6	7	8	14
137 A	Exp. 2 <i>a</i>	—	—	—	2	+ Exp. 3	
B	Tube 1	—	—	—	—	—	—
137 C	Exp. 2 <i>a</i>	1/10	10 R.T. 2.5	+	Dead		
D	Tube 2	1/10	20 R.T. 2.5	+	Dead		

Exp. 3*a*. Showing the result of Exposure 3.

Tube	Concentration of sodium thiosulphate	Source of trypanosomes	Number of trypanosomes per 256 squares of the haemocytometer scale at beginning of experiment	Condition of trypanosomes after 1 hour's exposure at 37° C.
1	1 : 10	Mouse 137A	5,000	All motionless
2	1 : 20	Exp. 2 <i>b</i> ...		Few feebly motile

TABLE IV—continued

Exp. 3*b*. Showing the result of injection of mice with trypanosomes after Exposure 3, and the resistance of the infection to reduced tryparsamide.

Mouse	Inoculum	Result of examination of blood of mice, and effect of treatment					
		Day after inoculation					
		3	4	6	7	8	9
171 A	Exp. 3 <i>a</i>	—	—	—	—	—	—
B	Tube 1	—	—	—	—	—	—
171 C	Exp. 3 <i>a</i>	—	—	5 R.T. 2·5	+	++	Dead
D	Tube 2	—	1/30	+ Exp. 4			

Exp. 22*a*. Showing the result of Exposure 22.

Tube	Concentration of sodium thiosulphate	Source of trypanosomes	Number of trypanosomes per 256 squares of the haemocytometer scale at beginning of experiment	Condition of trypanosomes after 1 hour's exposure at 37° C.
1	1 : 10	Mouse 571A	6,000	All motionless
2	1 : 20	Exp. 21 <i>b</i> ...		Some feebly motile

Exp. 22*b*. Showing the result of injection of mice with trypanosomes after Exposure 22, and the resistance of the infection to reduced tryparsamide.

Mouse	Inoculum	Result of examination of blood of mice, and effect of treatment					
		Day after inoculation					
		1	2	3	4	5	6
593 A	Exp. 22 <i>a</i> ...	—	—	1/3	+ R.T. 2·5	++	Dead
B	Tube 1						
	Exp. 22 <i>a</i> ...	—	1/20	15 R.T. 2·5	++	++	Dead
	Tube 2						

It appears from these experiments that a long series of exposures *in vitro* of an atoxyl-resistant strain to the maximum concentrations of sodium thiosulphate tolerated by the trypanosomes had no effect on the resistant character of the parasites.

Our experiments therefore fail to confirm the work of Citron, viz., that resistance to the aromatic arsenicals can be removed by sodium thiosulphate.

SUMMARY

Attempts made to influence the resistance of atoxyl-fast and novarsenobillon-fast strains of *T. rhodesiense* (a) by repeated administration of sodium thiosulphate to series of infected mice, and (b) by repeated exposure of these strains to concentrated solutions of sodium thiosulphate *in vitro*, were fruitless.

Consequently we were unable to obtain any evidence in support of Citron's conclusion that the resistance of a neosalvarsan-fast strain of trypanosomes can be abolished by the administration of sodium thiosulphate.

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THE POLYMORPHIC TRYPANOSOMES OF DAMBA ISLAND, VICTORIA NYANZA

II.—THEIR TRANSMISSIBILITY BY *G. PALPALIS*

BY

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The first paper of this series dealt with the ability of three strains of trypanosomes from the wild *G. palpalis* on Damba Island, Lake Victoria, to infect man.

The present contribution is concerned with the transmissibility of these three strains by laboratory-bred tsetse.

The three strains were examined by the usual method employed at Entebbe. Clean laboratory-bred flies were fed once or twice on the infected monkey, starved for 48 hours, and then fed on alternate days on a fowl until the 25th day after the first infecting feed. The flies were then fed on a clean animal, and finally dissected. Flies dying during the first few days of the experiment were rejected: the majority of these died without feeding, and in those that had fed any trypanosomes found may have been mere passive survivals in the undigested blood. All the other flies were dissected as they died. Each box of flies had a numbered fowl to itself.

The terms used in the column heads of the Tables are those originally employed in the Final Report of the League of Nations International Commission on Human Trypanosomiasis (1928). Briefly:—

$$\begin{aligned} \text{Metacyclic index} &= \frac{\text{Number of flies with gland infection}}{\text{Number of infected flies that lived 25 days or more}} \\ &= M, \end{aligned}$$

and *Transmissibility index* = $M \times$ total percentage of flies containing flagellates.

In the 'age' column, figures preceded by a * relate to gut-only infections, by a † to gut-and-gland infections.

The transmission experiments are given in Tables I and II. Table I gives the essential details of each separate experiment, and in Table II these details are summarised and constitute what may be regarded as the transmissibility formula for the strain.

These tests are sufficiently extensive to give a fair indication of the differences between the three strains. It will be seen that Strain 912 is more transmissible than the other two. Strain 912 ranks as a readily transmissible strain according to standards holding at Entebbe. It is considerably more transmissible than the very great majority of the *T. gambiense* strains freshly isolated from man that have been examined here during the last six years, and approaches more nearly the standard commonly attained by *T. rhodesiense* in *G. palpalis*.

Commentary on Table II.

A. The 1912 Damba antelope strain, isolated by the inoculation into a monkey of the blood of two young situtunga shot on the island in September, 1911 (Duke, 1912). These figures are, of course, too small to possess much importance beyond showing that the strain was transmissible by tsetse.

B. The 1920 strain, also isolated from the blood of situtunga shot on Damba (Duke, 1921).

C. The 1920 strain, after two years' upkeep by direct transmission at the laboratory (Duke, 1923).

D. The 1920 strain, after continued maintenance for a further 12 months by direct transmission (Duke, 1924). This affords a striking instance of complete loss of transmissibility by *G. palpalis* during prolonged maintenance by direct passage at the laboratory.

E. The 1926 strain, obtained by inoculation into a monkey of the blood of several situtunga shot on Damba (Duke, 1928). This interesting strain (No. XXIII) was tested in several different species of hosts, in all of which it proved non-transmissible by *G. palpalis*.

TABLE I

Transmission experiments with three strains of wild *G. palpalis* of Damba, 1932

No. of experiment	Strain	Day of experiment on which dissection began	No. of flies alive on 25th day	No. of flies dissected during the experiment			Duration of experiment, in days	No. of flies containing flagellates		Ages of the infected flies dissected during experiment	Dates of infecting feeds
				Males	Females	TOTAL		In gut only	In gut and salivary glands		
533	910	5	50	27	40	67	29	—	—	—	28 and 29.2.32
534	910	6	48	30	34	64	30	0	2	†26, †30	28 and 29.3.32
539	910	8	25	23	28	51	30	—	—	—	1 and 3.3.32
545	910	9	53	38	29	67	30	1	1	*30, †30	2 and 4.3.32
553	910	7	54	34	22	56	39	2	1	*20, *26, †39	5 and 7.3.32
554		9	49	33	22	55	38	0	0		5 and 7.3.32
569	910	9	27	27	22	49	40	0	1	†34	9.3.32
546	911	10	45	24	31	55	30	2	0	*10, *21	3 and 5.3.32
547	911	5	28	28	20	48	30	2	0	*12, *22	3 and 5.3.32
557	911	17	41	27	24	51	32	1	0	*22	6 and 8.3.32
558	911	8	33	29	20	49	32	2	1	*8, *13, †32	6 and 8.3.32
559	911	5	45	25	25	50	40	1	0	*8	7 and 9.3.32
560	911	9	44	28	23	51	40	0	0		7 and 9.3.32
529	912	10	43	22	31	53	34	4	1	*10, *34, *34, *34, †32	26 and 28.2.32
530	912	6	39	20	30	50	30	7	0	*6, *7, *11, *13, *13, *19, *30	27 and 29.2.32
531	912	5	31	25	20	45	31	3	1	*16, *17, *31, †31	27 and 29.2.32
537	912	6	51	31	33	64	30	1	1	*28, †30	1 and 3.3.32
538	912	5	53	18	39	57	38	0	1	†38	1 and 3.3.32
543	912	6	45	40	22	62	32	4	4	*6, *9, *14, *20, †32, †32, †32, †32	2 and 4.3.32
544	912	13	37	25	21	46	32	1	4	*13, †32, †32, †32, †32	2 and 4.3.32
556	912	11	35	18	22	40	40	0	0		6 and 8.3.32

TABLE II

Transmissibility index of the three wild *G. palpalis* strains of Damba, and, for comparison, of other strains previously isolated from *Tragelaphus spekei* on Damba

Animal	Date	Total flies dissected	Total infected flies	Percentage of infected flies	No. of infected flies alive on 25th day		Transmissibility index
					With glands infected	TOTAL	
910	28 Feb. to 9 Mar., 1932...	409	8	1.9	5	7	1.3
911	3 Mar. to 9 Mar., 1932 ...	304	9	2.9	1	1	2.9
912	26 Feb. to 8 Mar. 1932 ...	417	32	7.6	12	18	5.0
(1912 Damba) A	Nov. 1912... ..	179	3	1.6	1	2	0.8
(1920 Damba) B	Oct. 1920, to Jan. 1921 ...	731	17	2.3	5	7	1.6
(1920 Damba) C	Nov. to Dec. 1922 ...	426	12	2.2	0	9	0
(1920 Damba) D	Nov. to Dec. 1923 ...	977	0	0	0	0	0
(1926 Damba) E	Nov. 1926, to Mar. 1927	958	2	0.2	0	0	0

DISCUSSION

The total percentage of positive flies is perhaps the most useful feature of a test and is a figure unaffected by the accidents attendant on experiments of this nature. The significance of the figure called the *transmissibility index* depends upon the proportion of infected flies that live for 25 days or longer, i.e., long enough to acquire a gland infection if that is their destiny. It will be noted that this index cannot in any circumstances exceed the figure for the total percentage of positive flies: it may have little significance if the majority of the infected flies die young.

Of the three tests, that of 910 gives the truest estimate of the transmissibility of the strain. Seven out of the 8 infected flies lived 25 days. With the 911 strain, only 1 of the 9 infected flies lived the appointed period, and this showed a gland infection. The *metacyclic index* is thus unity, and the *transmissibility index*, in consequence, equals the infectivity percentage. Had more infective flies lived 25 days, the second index might have been considerably smaller. On the other hand, the *transmissibility index* of the 912

strain might well be greater than 5, as little more than half the infected flies survived the 25th day.

In deciding whether a strain is or is not cyclically transmissible by tsetse, the value of the test, apart from the actual number of flies employed, depends mainly on the proportion of the infected flies that live 25 days.* In the 911 test, for example, had no gland-infected flies been found, the fact that only 1 out of the 9 infected flies lived 25 days would have rendered the test practically valueless.

It must be understood that these indices are intended mainly for rough comparison between strains, and it has been found that, for this purpose, 'tests' of 400-500 flies are of real use.

SUMMARY

(1) One of the three 1932 strains of the Damba trypanosome, No. 912, was on its first isolation considerably more transmissible by *G. palpalis* than the other two strains.

(2) The transmissibility of this strain is higher than the majority of *T. gambiense* strains examined, and approaches the standard commonly reached by *T. rhodesiense* strains in the experiments carried out at Entebbe over a number of years.

(3) Strain 912 is more readily transmissible by *G. palpalis* than any other strain previously isolated from *Tragelaphus spekei* on Damba Island.

In a subsequent paper in this series, the animal reactions of the three 1932 strains and their response to human serum, to arsenic and to the red-cell adhesion test will be discussed.

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* The 25th day is chosen because experience has shown that in the conditions prevailing at Entebbe any fly destined to develop a gland infection will do so within this time. Some strains, particularly *T. rhodesiense*, reach the glands earlier, and in such cases the earliest date at which gland invasion is detected is used in the calculation of the indices.

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